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# BMJ Open

## ASSESSING POPULATION BASED SEROPOSITIVITY FOR ANTIBODIES AGAINST SARS COV2 IN AHMEDABAD CITY OF INDIA

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-044101
Article Type:	Original research
Date Submitted by the Author:	27-Aug-2020
Complete List of Authors:	Prakash, Om; Ahmedabad Municipal Corporation Solanki, Bhavin; Ahmedabad Municipal Corporation, Health Department SHETH, JAY; AMC MET Medical College, Community Medicine Joshi, Bhavin; Ahmedabad Municipal Corporation Kadam, Mina; AMC MET Medical College, Department of Microbiology Vyas, Sheetal; AMC MET Medical College, Department of Community Medicine Shukla, Aparajita; Smt NHL Municipal Medical College, Department of Community Medicine Tiwari, Hemant; Smt NHL Municipal Medical College, Department of Community Medicine Rathod, Sanjay; AMC MET Medical College, Department of Microbiology Rajput, Anil; AMC MET Medical College, Department of Microbiology Trivedi, Toral; AMC MET Medical College, Department of Microbiology Ramanuj, Vaibhav; Smt NHL Municipal Medical College, Department of Community Medicine Solanki, Anand; AMC MET Medical College, Department of Community Medicine
Keywords:	COVID-19, IMMUNOLOGY, VIROLOGY

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# **Title: ASSESSING POPULATION BASED SEROPOSITIVITY FOR ANTIBODIES AGAINST SARS COV2 IN AHMEDABAD CITY OF INDIA**

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Word Count

Abstract: 299

Article: 4370

# **Title: ASSESSING POPULATION BASED SEROPOSITIVITY FOR ANTIBODIES AGAINST SARS COV2 IN AHMEDABAD CITY OF INDIA**

## **ABSTRACT**

Objectives: To study the percentage sero-positivity for SARS-CoV2 to understand the pandemic status and predict the future situations in Ahmedabad

Study Design: Cross Sectional study

Settings: Field area of Ahmedabad Municipal Corporation

Methods: A large scale sero-surveillance with more than 30,000 samples was carried out in the Ahmedabad, Gujarat, India. Enrolled participants included general population including HCWs from 75 Urban Primary Health Centers (UPHCs) across 48 wards and 7 zones of the city. Study included various group of people irrespective of age, sex, acute/past covid19 infection. The health department had collected the data using a simple brief format with minimum basic primary demographic information. We analyzed the data, calculated seroprevalence and tried to correlate seropositivity with various factors for valid and precise predictions on the herd immunity status of the population.

Results: With seropositivity between 15%-20% for various age groups, the crude seropositivity is 17.61%. The difference in seropositivity for both sex groups is not significant. The seropositivity is significantly lower(13.64%) for Health Care Workers (HCW) as compared to non-Health Care Workers (18.71%). Week wise seropositivity shows increasing trend with time. Zone with maximum initial cases have high positivity as compared to other zones. UPHCs with

recent rise in cases are leading in seropositivity as compared to earlier and widely affected UPHCs.

Conclusions: The herd immunity status is still low and the population of Ahmedabad is still largely susceptible. Presently, we cannot rely on the herd immunity to protect and the preventive measures need to be strongly relied upon till an effective vaccine is available to the people at large. The data indicate the possibility of vanishing immunity over time and need further research to cross verify with scientific evidences.

Key words: SARS-COV2; Covid-19, Serosurveillance; Herd immunity

**Strengths and Limitations of the study**

- The sample is representative to the population and a very large sample size, probably one of the largest sample population ratio to find out seropositivity.
- Interesting scientific findings for the seropositivity for SARS-COV2
- Health Care Workers also included in sample.
- Seropositivity also compared with existing confirmed case load
- Only few factors considered for checking correlation of seropositivity.

## Introduction:

A new respiratory virus causing severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) was first reported from China in December 2019 and soon spread throughout the world.<sup>1,2</sup> The world health organization (WHO) declared it as Pandemic and named the disease caused by this virus as COVID-19.<sup>3</sup> Being a newly identified virus, the scientific community is largely unaware of the natural history and the immune response developed after the Covid19 infection.<sup>4</sup> Since the virus is novel in origin, the initial seroprevalence in the population is assumed to be negligible. During the process of recovery from an infection, antibodies are developed, the presence of which may be tested to mark the presence or absence of an immune response. Serological survey is one of the most widely used tools to identify the presence of these antibodies which develops as a result of an immune response to an infection. A population based sero-survey can help in estimating cumulative incidence of infection as well as extent of the infection in the community.<sup>5</sup> WHO also recommends monitoring of sero-prevalence over time for anticipating disease dynamics and planning an adequate public health response.<sup>6</sup> The sample size in such sero sample survey should be large enough to get reliable parameters sufficient enough to draw conclusions and future public health actions.<sup>7</sup>

Serological tests and the sero-epidemiology greatly helps in understanding the disease transmission, population susceptibility as well as the public health measures to be followed.<sup>8</sup> Since, the test for Covid19 infection turns out to be positive even in an asymptomatic patient, restricting the serological testing only to symptomatic individual will not give a real picture.<sup>9</sup> On the other hand a field level population based testing will give a better assessment of disease situation and the specific immunity following its infection. While the positive results indicate

what proportion of the testing population has developed complete or partial immunity, those with negative result gives hint about the proportion of susceptible population.

India being the second most populous country with high population density is at high risk from covid19 pandemic. Ahmedabad city was among the first few cities severely affected by the spread of the pandemic. Ahmedabad city of India, having 7 million population and more than 16,000 Covid-19 cases before starting of this study, one of the earliest cities to witness the high case load in the initial months of the pandemic in India, Ahmedabad is ideally suited to study the percentage sero-positivity to help understand the pandemic status and predict the future situations.

**Aim:**

- To analyse and study the available data related to Covid-19 sero-positivity in Ahmedabad City

**Objectives:**

- To calculate the sero-prevalence of Ig-G antibodies to COVID-19 in the general population in Ahmedabad
- To correlate the sero-positivity with various factors for better understanding of the pandemic situation
- To identify the status of herd immunity for valid & precise predictions for the future.

## Methodology:

Looking to the monitor the pandemic, understand its present situation and to take appropriate corrective public health measures, the Indian Council of Medical Research (ICMR) issues directives to all the state governments to carry out IgG Elisa test for sero-surveys along with ILI & SARI Surveillance. The primary purpose of this was to understand the proportion of population exposed to SARS-CoV-2 infection including asymptomatic individuals. The Health department of the local municipality of Ahmedabad – Ahmedabad Municipal Corporation (AMC), from the state of Gujarat, INDIA, carried out a large scale population based serological survey for Ig G antibodies against SARS-CoV-2 Virus. This was the first ever primary situational analysis of the immune status against the SARS CoV2 infection from India. “Covid Kavach” (Anti-SARS CoV-2 Ig G Antibody Detection ELISA) kits developed and manufactured by Zydus Diagnostics, validated by National Institute of Virology, Pune, India and Approved for use by the Indian Council of Medical Research (ICMR) was used for the purpose of this study after due approval. As per the validation reports, the kits have a sensitivity of 92·1% and a specificity of 97·7%. So, with very high level of sensitivity and specificity it may be noted that the results received through this testing kit is highly reliable.

As there were reports of asymptomatic infections in Covid19, it was important to consider sampling beyond the symptomatic cases. Authorities of AMC preferred a field level serological study over case load or case density related sampling to get the real status of immunity. In a serological survey, it is utmost important to separate out the serum which cannot be done without specific laboratory equipment. This was the reason why the study cannot be done by house to house survey. To avoid sample rejection in such sero survey, what is crucial is that the collected

1  
2  
3 blood sample is handled carefully with minimal shaking and that too subjected for serum  
4 separation as soon as possible. This is the reason why the blood sample collection and serum  
5 separation was done at the same place at the Urban Primary Health Centre, where a trained  
6 laboratory technician is available along with necessary basic laboratory support for handling the  
7 sample and separating the serum. At the end of the day all the serum samples were sent to the  
8 designated laboratories for the purpose of testing.  
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19 Ahmedabad city is divided into 48 wards distributed across 7 zones. There are 75 UPHCs which  
20 cater the primary health care services to the local population. To get a real picture of the existing  
21 immune status, the convenience sampling was followed and sample population was selected  
22 from all the UPHCs irrespective of high/low case load or color categorization of the Covid19  
23 risk based on the Aarogyasetu Application data. Individuals were enrolled at the level of UPHC  
24 without any exception. An effort was made to cover a wide variety of people of different age  
25 groups from both the gender and falling into various categories such as cases, close contact of  
26 cases, super-spreaders, symptomatic individuals, asymptomatic individuals, patients attending  
27 OPD for other ailments, general population as well as field level healthcare workers. Population  
28 in the UPHC served area were informed about such survey by the field level health care workers  
29 and all the willing individuals were invited to get enrolled. The field workers facilitated their  
30 visit to the UPHC and enrollment. At times parents wished and requested that their children be  
31 tested. In such cases their request was respected and even minors were enrolled in the study.  
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33 Thus the inclusion criteria allowed inclusion of all irrespective of age, sex, acute/past covid19  
34 infection. Exclusion criteria included refusal to give informed verbal consent or any  
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3 contraindication to venipuncture. Considering an average roughly estimated 60 lakh population,  
4 and a target of 30,000 tests, approximately 0.5% of the city population was covered in this study.  
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10 Ahmedabad Municipal Corporation manages 1 dedicated Covid19 hospital which is attached  
11 with a medical college. There are 2 other non-Covid hospitals which are attached with medical  
12 colleges and run by the corporation. Many of the health workers working at these hospitals have  
13 developed covid19 infection, irrespective of the Covid status of the hospital. Authorities were  
14 concerned about the immune status of these health care workers and invited willing health care  
15 workers from the 3 hospitals under their administrative control to participate. So, apart from the  
16 75 UPHCs, enrollment of the health care worker was also carried out at these 3 hospitals for the  
17 purpose of antibody testing.  
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31 Since the enrollment in the study involved collection of blood for the purpose of testing  
32 antibodies, an informed verbal consent was taken from all the participants before enrollment.  
33 Strict confidentiality was ensured at all the levels. Looking to the available test kits and the  
34 capacity of the UPHC laboratory for handling the samples and conducting the serum separation,  
35 the daily capacity of samples per UPHC was limited. So, the study continued over a period of  
36 approximately 3 weeks. For the purpose of testing and standardization, only those laboratories  
37 attached with a medical college with all necessary equipments and facilities were considered.  
38 Since these medical college laboratories were also involved in RT-PCR testing for the Covid19,  
39 the antibody testing capacity of these medical college laboratories was also limited. So, to cope  
40 up the need for timely testing of the bulk sample for antibodies, private laboratories with national  
41 level accreditation and state of the art facilities and equipments were invited to participate and  
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support. Finally, a total of 5 such private laboratories supported in testing the samples apart from 2 laboratories attached with a medical college.

To standardize the sample collection and testing, a brief demonstration session cum training covering all necessary information was carried out by the concerned health authorities either at zonal level or sub-zonal level with appropriate safety and social distancing measures. All personnel involved in the study were trained for standard as well as droplet precautions as part of infection prevention and control procedures particularly with reference to the Covid19 as per the national guidelines

AMC had collected the data of the enrolled participants in a simple brief format with minimum basic primary demographic information. This data was collected at the UPHC level for all the enrolled individuals and it was compiled at the zonal level and shared with the health department on daily basis. Simultaneously all the collected samples were delivered at the pre-decided laboratories for timely testing and reporting. The dataset was updated routinely on declaration of the results. An in-depth analysis of available data was crucial for valid and precise estimation of immunity status and for guiding the authorities for taking appropriate public health measures in a timely manner. So, the faculty members involved in corona control room activities, field monitoring & supervision as well as antibody testing were invited to critically analyse the available data and draw scientific conclusion on the level of herd immunity. We tried to find out the crude positivity as an indicator for the level of herd immunity as well as the factors affecting it. We shared the results with the concerned authorities to take appropriate public health

measures for larger benefit of the population. We herewith share the results for the detailed insight by the scientific community.

Patient and Public Involvement: patients were not directly involved in the planning or implementation of the sero surveillance. However, these are carried out by the administrators of the local self governance (Municipal Corporation) and was carried out for their benefit and determining herd immunity status. The results were declared to the local media through press conference and were widely publicized on social media through executive summary and published by local daily news papers as well.

**Results & Discussion:**

A total of 30054 blood samples were collected from the Ahmedabad city for the purpose of the study. The present study is one of the biggest studies conducted in the world in terms of sample population ratio to find out sero positivity in general population. This study has 4770 samples per million population in comparison to Spanish study where the sample population ratio was 1302 samples per million population and the US study where the sample population ratio was 255 samples per million population.<sup>10,11</sup>

Out of the total 30054 samples 1511 samples were collected from the 3 hospitals while the remaining samples were collected from the 75 UPHCs. Out of the total samples, 163 samples were not tested / rejected for various reasons. Results were available for the remaining 29891 samples (Figure 1). A total of 24197 tests were Negative while 431 test results were reported as indeterminate. Thus, a total of 5263 results were positive for the specific antibodies against Covid19 giving an overall crude positivity of about 17·61%. Our results of sero-prevalence are consistent with other studies showing that even in the areas highly affected by SARS CoV2 during this pandemic, have shown very low level of seropositivity.<sup>8</sup>

There were 16135 males and 13919 females enrolled in the study and results were available for 29891 individuals. A total of 2774 from 16044 males were tested positive giving the positivity rate of 17·29%. A total of 2489 out of 13847 females were tested positive giving the positivity rate of 17·98%. Thus the percentage positivity is slightly higher among females as compared to the males but the difference is statistically not significant ( $P > 0.05$ ). This finding is similar to

other studies from Spain & USA where the studies have found no significant difference between the genders for covid19 antibodies.<sup>7,8,12</sup>

The age wise analysis shows that the age of the sample population range from <1 year to 100 years with an average of 39·10 years with a standard deviation of 14·54 years. Among the sample, the mean age of females is 37·70 years with a Standard Deviation of 13·94 years, whereas the mean age of males is 40·25 years with a standard deviation of 14·88 years. Considering the sero-positive, the mean age for females is 38·69 years with a SD of 14·16 years where as that of male is 41·41 years with a SD of 14·51 years.

The age group wise analysis of total tests and positive tests when compared to calculate percent positivity (Figure 2) shows that the positivity in various age-groups is between 15-20 percent. Considering the gender variation also for different age groups (Figure 3), the percent positivity for both the genders is seen between 15 to 20 percent for most of the age-groups. The agewise distribution is statistically significant, as the positivity at the extremes of age group i.e. 0-9 years and 90-99 years for both the gender groups varies. This may be on account of very less individuals in the sample from these age-groups as can be verified in table-1.

The zone wise positivity shows that the zones most affected by covid19 i.e. Central zone with maximum initial cases had the highest positivity of 28·43%. Thus the positive antibody status very well correlates with the documented evidence of high load of cases particularly in the first 2 months of the pandemic in Ahmedabad. Antibodies do take some time to develop after an infection, approximately 1 to 3 weeks, with an average of 2 weeks (14 days).<sup>13,14</sup> So, we can say

that the rate of antibody positivity reflects the case scenario about 14 days prior to the study. Since majority of the samples were collected by 30<sup>th</sup> June, we can check the results with the clinical case scenario seen about 14 days before i.e. 16<sup>th</sup> June 2020.

In the present study there were 6509 health care workers. There were 888 sero positive out of these HCWs giving a sero positivity of 13·64%. On the other hand there were 4375 seropositive individuals from the remaining 23382 who were not the health care workers and resulting into 18·71% sero positivity among these non HCWs. This difference is statistically significant and implies that HCWs have an overall low sero-positivity as compared to general population because they are usually better protected as compared to the general population.

Looking at the UPHC wise analysis (Table-2), it is seen that the UPHCs with higher case load have higher positivity but there are variations also. UPHCs with high case load in middle of April and middle of May month have lesser positivity compared to the UPHCs with higher case load in middle of May to middle of June 2020. In Ahmedabad, the initial cases were more from the central zone. Then, gradually due to extensive containment measures the cases from central zone reduced and then cases started appearing more from the East and North zones. It can be seen that the highest seropositivity was found in the individuals from East Zone and North Zone. This is on a slightly higher side than that from the UPHCs of the Central Zone, which had the highest cases. Strikingly the worst and first affected UPHCs of the Central zone have lesser positivity. The first 8 UPHCs with the highest positivity are from North Zone & East Zone (Having higher cases in the later part of the pandemic period so far) than that of Central zone (having maximum population density and highest cases in the very first phase of pandemic

period). This might be pointing towards the fact that the immunity developed after a successful recovery from Covid19 infection may not be lasting enough. We need more studies and longer follow up to cross verify this aspect and to bring out the scientific fact related with the post-covid immunity.

Looking at the day to day positivity (Figure 4), it was found that there is some variation. This is partially due to the fact that different population groups were primary target for different days so as to cover a wide variety of groups (e.g. cases, controls, super spreaders, symptomatic, asymptomatic, health care worker etc). Representation of all such group collectively makes the sample quite representative to the population of the city. Checking the trend of positivity by adding a linear trendline, we can say that gradually there is increasing trend, which can correlate with the increasing number of cases (14 days before) presenting with antibody positivity.

As can be seen from Table-1 the seropositivity of the serum samples from first, second and third week of the study is calculated to be 13·83%, 20·20% and 22·33% respectively. This shows that the positivity increase with time & it correlates very well with increasing number of cumulative cases, who develop immunity on account of their past infection. This increase in seropositivity in week 2 is independent of any area as it shows similar high positivity for all the 7 zones. To neutralize any effect of high positive on any particular day, the cumulative positivity on weekly basis was analysed (Figure 5). The seropositivity of combined 2 weeks shows higher positivity than week 1 and this trend is consistently seen for all the zones. Continuing the same trend, when the combined positivity of first 3 weeks (completed study) is compared with the positivity of first 2 weeks, it does not show any significant variation. The percentage positivity almost remain

similar. This may be due to comparatively lower number of sero samples collected during the third week.

Zone wise comparison of cases per 10000 population and percent positivity can be seen in Table-3 & Figure 6. While trying to compare with the seropositivity, it is seen that the picture is well correlated in most zones. The comparative low positivity as compared to high case per 10,000 populations may be due to largely equitable distribution across zone since the enrollment and sampling at UPHC level and Zone level was not according to the case load. However, it is being observed that the sero positivity is comparatively lower in the areas of old infection than areas of recent infection (Figure 7). For example, the first and worst affected zone with highest population density as well as case density (214 cases per square kilometers) is showing only 28.43% sero positivity. This is also true in case of South zone which was second worst affected is also showing sero positivity of 16.15 % only. Areas of recent infection like North Zone and East zone are showing better sero positivity of 27.42% and 23.22% accordingly despite having case density of 44.5 and 34.6 cases per square kilometers respectively.

When we compare cases per million population and sero positivity it was found that zones who were affected in initial days (South zone and Central zone) having more cases per million population but that is not being reflected in terms of sero positivity. Central zone having 5743 cases per million population is having 28.4% zero positivity and South Zone having 3028 cases per million population is having sero positivity of only 15.89% but areas of recent infection like North Zone and East zone are having higher sero positivity (44.5% and 34.6% respectively) in comparison to south zone despite lower number of cases per million population. This gap may be

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3 indicative of vanishing positivity with the passing of time. However, we need more indepth  
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5 scientific research to find out the reason behind this paradox.  
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10 Zone wise distribution of the individuals who were tested for the Antibodies according to their  
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12 age group shows that the age wise distribution was following similar trend in all the zones.  
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14 While the middle age groups (20-30 & 30-40) were the age groups with highest number of tests  
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16 for all the zones. However, if we compare the same trend (age-group and zone wise) for the sero-  
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18 positive individuals, then the peak is slightly on the right side (higher age group, i.e. 30-39 and  
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20 40-49 years) This also correlates with the zone independent age group wise positivity, which  
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22 shows the peak of the test positivity at the 40-49 years age group  
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28 Looking at the positivity rate at the Covid/Non-Covid hospitals, it is observed that the highest  
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30 positivity (12·84%) is seen at the SVP hospital which is a dedicated Covid hospital right from  
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32 the start of the corona pandemic in the city. The overall positivity of 12·84% is still lower than  
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34 that of the crude positivity of the general population i.e. 17·61%. This may be due to the fact that  
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36 all the health care workers in a dedicated Covid hospital are very well protected with PPE &  
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38 other safety measures. SVP hospital is also one of the state of the art paper-less hospital with the  
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40 latest technologies to support the infection prevention and control (IPC) measures.  
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47 LG hospital is the other non-covid hospital attached with another medical college run by the  
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49 Ahmedabad Municipal Corporation. The positivity rate at LG is also quite similar to SVP  
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51 hospital with 12·80% positivity. Although LG hospital is not a dedicated covid hospital still the  
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53 positivity rate here is quite similar to that of the covid hospital. However, if we try to correlate  
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the situation of this hospital with the positivity, it can be explained very easily. Inspite of being a non-Covid hospital, there were multiple occasions in the previous 2 months when large number of health care workers turned out to be positive & were isolated/quarantined. A lot of patients from the containment zone reported here for non-covid complains but were tested & reported positive. The authorities were even forced to close down the hospital once for about 7 days for improving the sanitization measures and improving the implementation of various SOPs. As compared to the above two hospitals, the other non-covid hospital (SCL Hospital) did not have many Covid cases and the low positivity (3.09%) at this hospital also correlate well with the data.

Comparing the positivity for Health care workers and non-Health care workers among the close contacts (Figure 8), it is clearly seen that the positivity among the health care worker is lower than that of non-health care worker. This is true across all the age groups. This can well be correlated with the fact that the health care workers are better protected when being a close contact of a confirmed case where as non health care workers are usually exposed with no or minimal protection. Overall, the percentage positivity was higher among the field level health care workers as compared to the HCW from the hospitals. This may be due to the fact that the HCW from the hospital are often better protected due to the clarity of the risk involved whereas the field level health care workers are often poorly or partially protected with PPE and other protective measures.

## Summary:

This study to assess the seropositivity after covid19 infection from Ahmedabad, India is one of the first few population based study from India with a very large sample size. This sero-positivity against Covid19 in Ahmedabad is around 17.61%. This indicates overall low level of herd protection as of now. A majority of the total population has not yet suffered from the disease and has not yet developed the immunity and is still largely susceptible. The preventive measures must be strongly followed for continued control situation of the pandemic disease in the city.

The sero-positivity for various age groups is seen between 15% to 20%. The level is consistent for both the genders without any significant difference. The sero-positivity is significantly lower (13.64%) for HCW as compared to non-HCW (18.71%).

The zone wise positivity closely correlates with the cases recorded so far, but there is a wider gap between case-load as well as Case-density and the sero-positivity for the earliest and worst affected zones (Central zone followed by South zone) as compared to the zones affected recently. The UPHC wise positivity is also seen higher for the UPHCs which have comparatively higher cases in recent times as compared to UPHCs with higher case load at the beginning of the pandemic. Both of these might be pointing towards vanishing immunity over time and need further research to cross verify with scientific evidences to prove this observation.

Tracking the day to day positivity & weekly cumulative positivity shows an increasing trend over time. The positivity at the non-Covid hospital is lower than that of Covid hospital, but the non-covid hospital with multiple known incidents of covid transmission has almost similar rates

as that of Covid hospital. Overall positivity in Covid/non-covid hospital is lower than that of general population. As close contacts, HCW have low sero-positivity as compared to non-HCWs and within the HCWs, the field level HCW have higher seropositivity as compared to hospital based HCWs who are generally better protected.

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## Conclusion:

This seems to be the biggest ever study conducted in the world in terms of sample population ratio to find out sero positivity in general population. This study has 4770 samples per million population in comparison to Spanish study “Prevalence of SARS-CoV-2 in Spain (ENE-COVID): a nationwide, population-based sero epidemiological study” where the sample population ratio was 1302 samples per million population and US study “Seroprevalence of Antibodies to SARS-CoV-2 in Six Sites in the United States” where the sample population ratio was 255 samples per million population.

At present, the low level of Covid-19 seropositivity of 17.61% in Ahmedabad city, points out overall low level of herd protection as of now.

There is no gender difference in sero-positivity but the seropositivity is significantly associated with the risk of covid-19 infection in the area & occupation. Although the sero-positivity slowly increases with time there are also indications that the immunity may not be long lasting. In view of these findings with the absence of an evidence of lifelong immunity after Covid-19 infection, it can be concluded that the population of Ahmedabad is still largely susceptible. As of now, we cannot rely on the herd immunity to protect and the preventive measures need to be strongly relied upon till an effective vaccine is provided to the people at large.

Further in depth scientific studies are required to give more insight for the future predictions.

**Research in context**

**Evidence before this study**

Although the scientific community is aware of the general immune response after any viral infection, owing to its novel origin, there is very little information about the immune response after Covid-19 infection.

**Added value of this study**

Our findings at 3 months after the first case of Covid-19 in Ahmedabad, shows that inspite of very high number of cases, the overall seroprevalence is still low. There are indirect indications that there may be vanishing immunity over a time (2-3 months). The detailed analysis shows how the virus is affecting both genders equally and across different age groups

**Implications of all the available evidence**

The low level of crude seropositivity from the present study, suggest that population is still largely susceptible. We still need to apply public health preventive measures for effective control of the pandemic situation and till an effective vaccine is available for the mass. The results also indicate the scopes for further research to confirm and generate evidences regarding the vanishing immunity over a period of time.

**Author Contributions:**

Dr. Bhavin Solanki (BS) under the guidance of Dr. OM Prakash (OP) planned and carried out the serosurvey. Testing of the samples and reporting was managed by Dr. Sanjay Rathod (SR), Dr. Anil Rajput (AR) and Dr. Toral Trivedi (TT) under the leadership of Dr. Meena Kadam (MK).

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2  
3 Data analysis was carried out by Dr. Jay Sheth (JS), Dr. Sheetal Vyas (SV), Dr. Aparajita Shukla  
4 (ASh), Dr. Bhavin Joshi (BJ), Dr. Vaibhav Ramanuj (VR) and Dr. Anand Solanki (ASo) while  
5  
6 the the statistical analysis was done by Dr. Hemant Tiwari (HT). Primary manuscript was  
7  
8 prepared by Dr. Omprakash (OP) and Dr. Jay Sheth (JS) and equally contributed by all the other  
9  
10  
11  
12 coauthors. All authors contributed to the interpretation of data and approved the final manuscript  
13  
14  
15 after critical review.  
16

### 17 18 19 **Declaration of interests:**

20  
21 We declare no competing interests.  
22  
23  
24  
25

26 **Funding:** Health Department of Ahmedabad Municipal Corporation carried out the study as part  
27  
28 of Covid19 pandemic response on its own. There are no external funding  
29  
30  
31

### 32 33 **Data sharing:**

34  
35 Our data are accessible to researchers upon reasonable request for data sharing to the  
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37 corresponding author.  
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39  
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41

### 42 43 **Ethical permission:**

44 We have received permission from the Institutional Review Board for the study.  
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48

### 49 50 **Acknowledgments:**

51 We are extremely thankful to respected Dr. Rajiv Kumar Gupta, IAS (Additional Chief  
52  
53 Secretary, Government of Gujarat) and Mr. Mukesh Kumar, IAS (Municipal Commissioner,  
54  
55 Ahmedabad) for their whole hearted support. This study would not have been possible without  
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the financial support from the authorities of Ahmedabad Municipal Corporation. We acknowledge the full support from the field level health care workers (Corona warriors) who put in great efforts to perform their duties as well as sample collection after informed verbal consent particularly in a Covid-19 pandemic situation. All the Zonal Deputy Health Officers, Deputy Health Officer (Epidemic), Assistant Health Officers and Medical officers of the Urban Primary Health Centers extended full support in conducting the sero-surveillance. We are thankful to all the medical and paramedical support staff posted at the laboratories for their contribution in timely testing such large amount of samples with accuracy and quality. Finally, we are indebted to all the patients including health care workers whose willingness and support has generated the much desired data for the study.

Table-1 Analysis of Covid19 sero-survey positivity

	Female			Male			Total			P Value
	Results	Positive	% Positivity	Results	Positive	% Positivity	Results	Positive	% Positivity	
Gender	13847	2489	17.98%	16044	2774	17.29%	29891	5263	17.61%	P>0.05
Age groups										
0-9	42	3	7.14	53	14	26.42	95	17	17.89	P < 0.001
10-19	551	92	16.70	687	106	15.43	1238	198	15.99	
20-29	4011	684	17.05	3665	518	14.13	7676	1202	15.66	
30-39	3424	569	16.62	3910	636	16.27	7334	1205	16.43	
40-49	2837	528	18.61	3162	668	21.13	5999	1196	19.94	
50-59	1749	356	20.35	2608	499	19.13	4357	855	19.62	
60-69	889	189	21.26	1356	222	16.37	2245	411	18.31	
70-79	291	60	20.62	505	95	18.81	796	155	19.47	
80-89	49	8	16.33	93	14	15.05	142	22	15.49	
90-99	4	0	0	5	2	40.00	9	2	22.22	
Zone										
CZ	1867	560	29.99	1896	510	26.90	3763	1070	28.43	P < 0.001
NZ	2216	578	26.08	2569	734	28.57	4785	1312	27.42	
EZ	1603	366	22.83	2027	477	23.53	3630	843	23.22	
SZ	1493	251	16.81	1627	253	15.55	3120	504	16.15	
SWZ	1141	165	14.46	1838	235	12.79	2979	400	13.43	
WZ	3190	351	11.00	3108	310	9.97	6298	661	10.5	
NWZ	1356	75	5.53	2301	160	6.95	3657	235	6.43	
Hospital										
SVP (Covid)	339	37	10.91	331	49	14.80	670	86	12.84	P < 0.001
LG	418	55	13.16	207	25	12.08	625	80	12.80	
SCL	101	1	0.99	93	5	5.38	194	6	3.09	
Study week										
Week1	6535	942	14.41	6654	882	13.26	13189	1824	13.83	P < 0.001
Week2	5840	1208	20.68	7826	1553	19.84	13666	2761	20.20	
Week3	1472	339	23.03	1564	339	21.68	3036	678	22.33	
Category										
Non-HCW	10114	2003	19.80	13268	2372	17.88	23382	4375	18.71	P < 0.001
HCW	3733	486	13.02	2776	402	14.48	6509	888	13.64	

Table-2 Top ten UPHC with highest seropositivity results

UPHC of AMC	Total	Results	Positive	Positivity
MEGHANINAGAR (NZ)	119	119	52	43·70
GOMTIPUR (EZ)	308	300	118	39·33
RAJPUR (EZ)	383	383	138	36·03
NARODA ROAD (NZ)	170	170	61	35·88
INDIACOLONY (NZ)	806	802	280	34·91
BAPUNAGAR (NZ)	316	316	110	34·81
KUBERNAGAR (NZ)	480	477	166	34·80
SARASPUR-RAKHIAL (NZ)	401	401	127	31·67
JAMALPUR (CZ)	429	428	135	31·54
SHAHPUR (CZ)	394	394	124	31·47

Table-3 Zone wise comparison of seropositivity and Covid19 cases

Zone	Population	Area (Sq Km)	Cases (As on June 15, 2020)	Case Density	Cases per 10000 population	Results	positive	Percent positivity
CZ	683089	18.33	3923	214.0	57.43	3763	1070	28.43
SZ	1081996	88.65	3276	37.0	30.28	3120	504	16.15
NZ	1090409	64.92	2889	44.5	26.49	4785	1312	27.42
EZ	1425254	73.2	2532	34.6	17.77	3630	843	23.22
WZ	1302500	65.68	2357	35.9	18.10	6298	661	10.50
SWZ	524970	61.86	871	14.1	16.59	2979	400	13.43
NWZ	807539	91.36	772	8.5	9.56	3657	235	6.43

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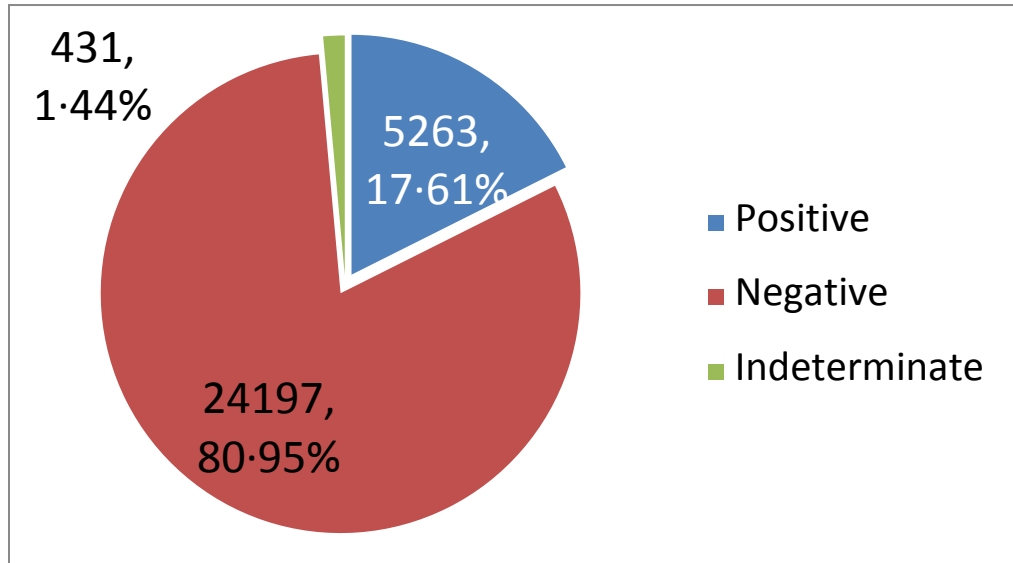


Figure1: Results of sero samples tested

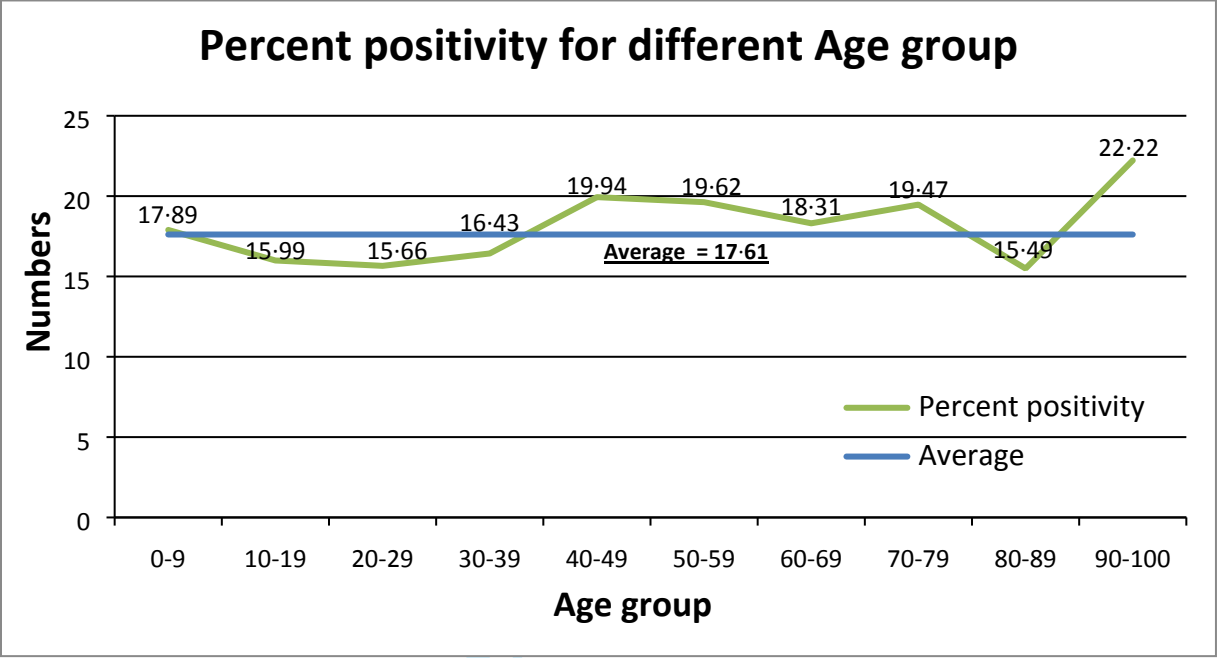


Figure 2: Age group wise sero-positivity in Ahmedabad

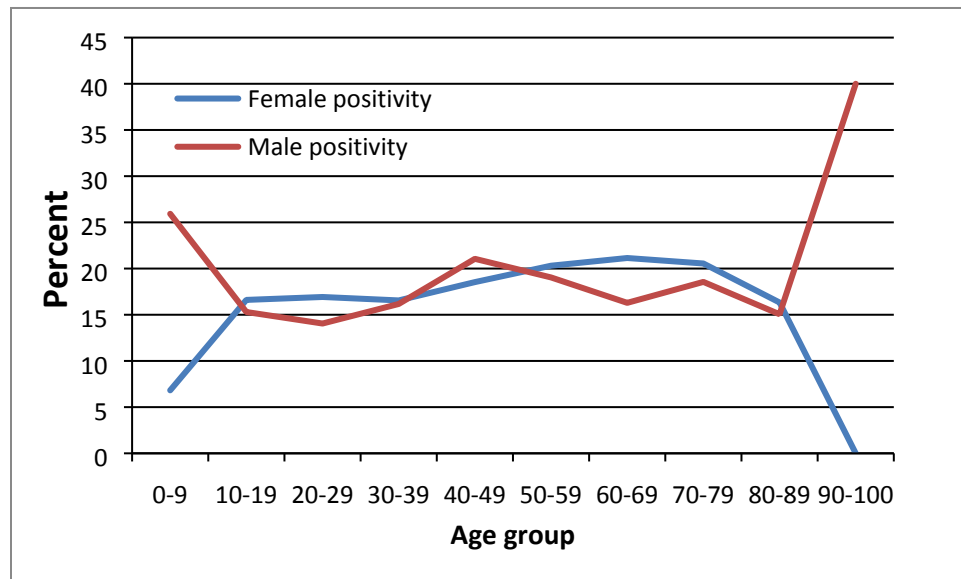


Figure 3: Age group & sex wise sero-positivity in Ahmedabad

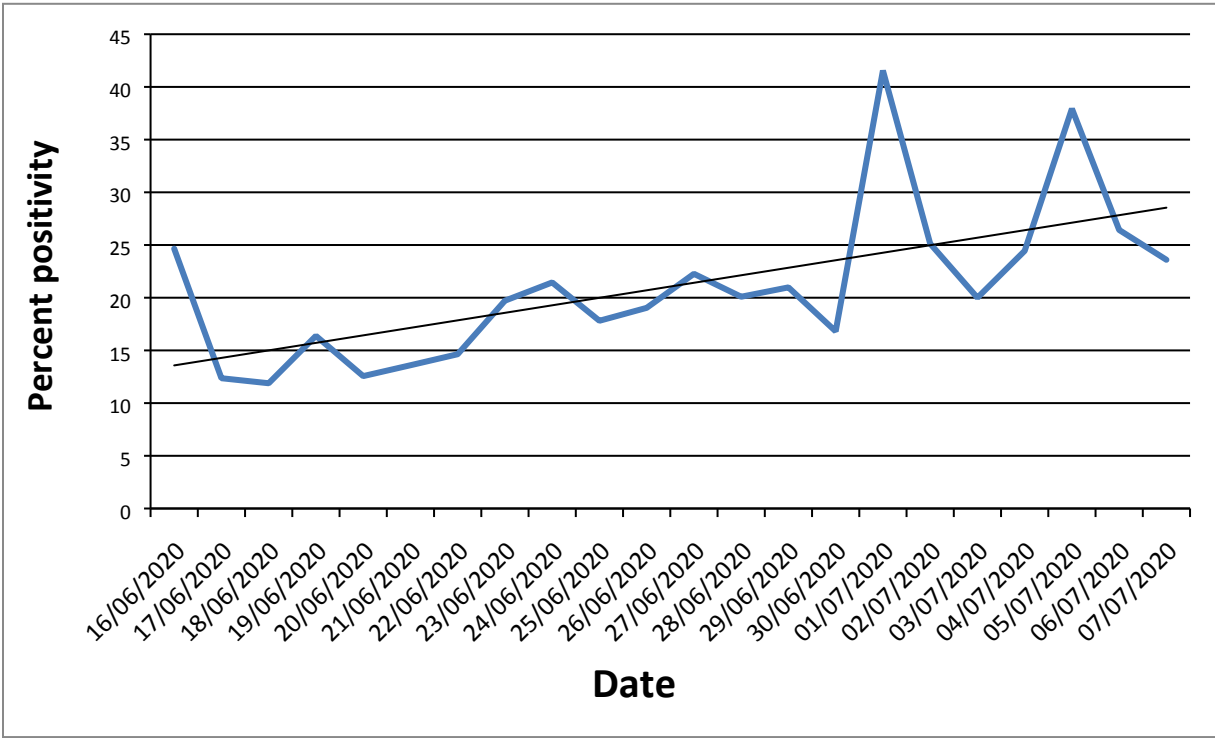


Figure 4: Trend of day to day sero-positivity in Ahmedabad

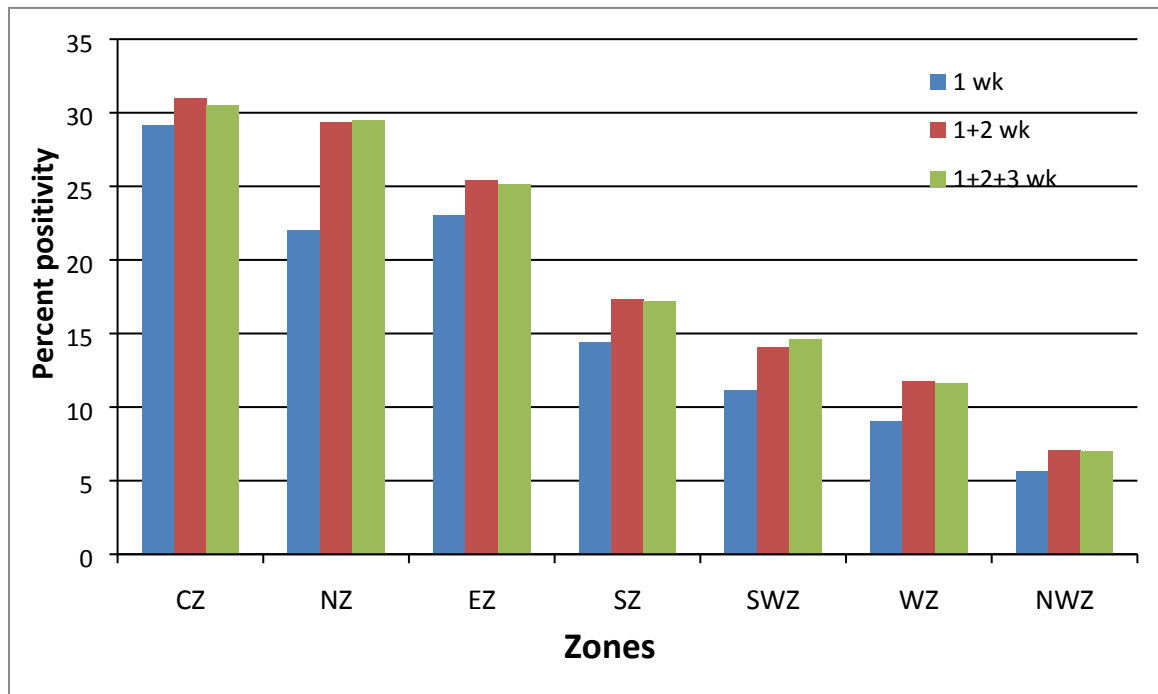


Figure 5: Week wise seropositivity trend for different zones in Ahmedabad

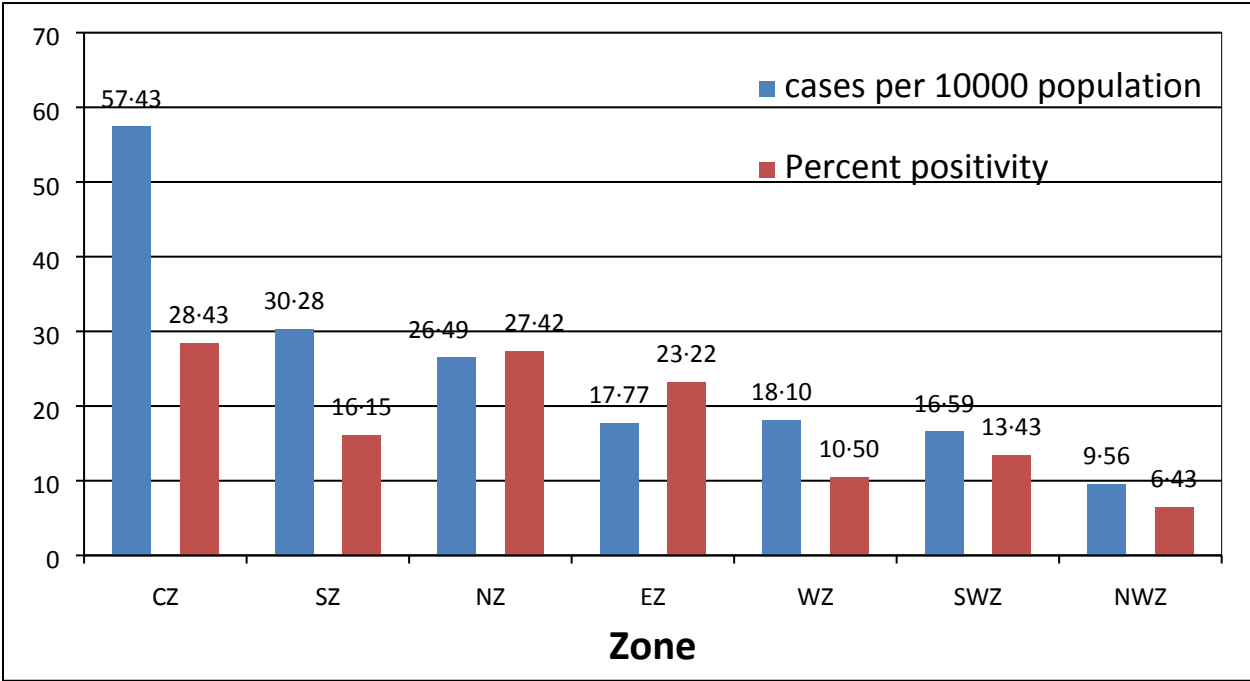


Figure 6: Zone wise case per 10000 population and Covid19 seropositivity

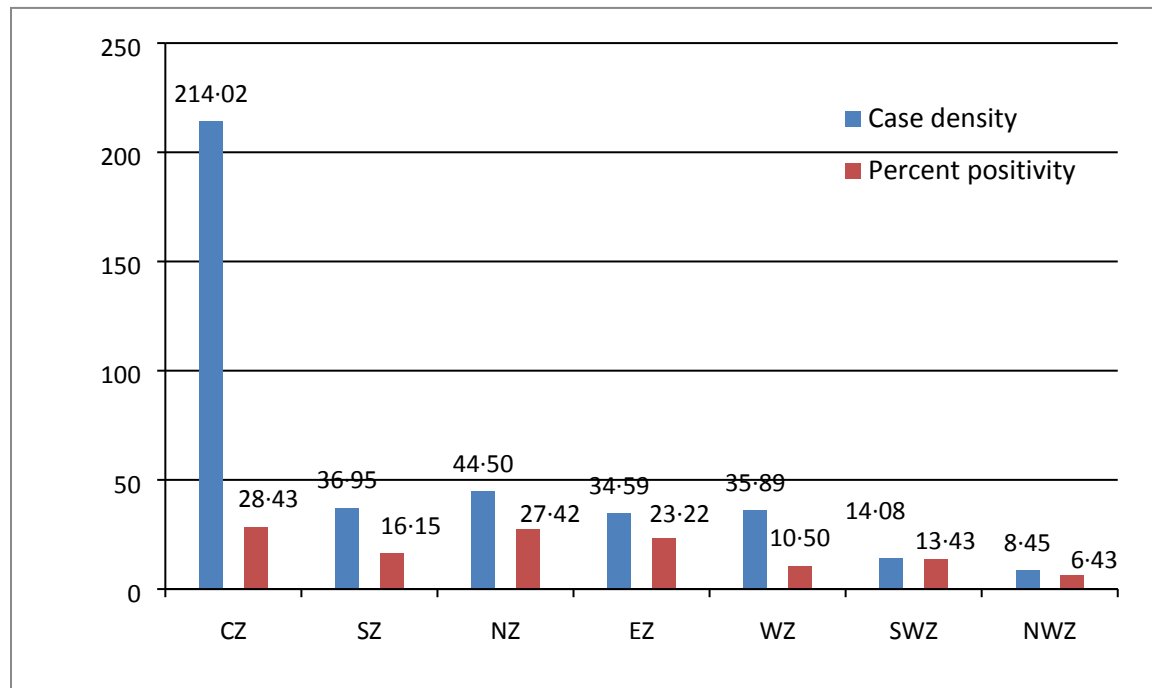


Figure 7: Zone wise comparison of seropositivity and Covid19 case density

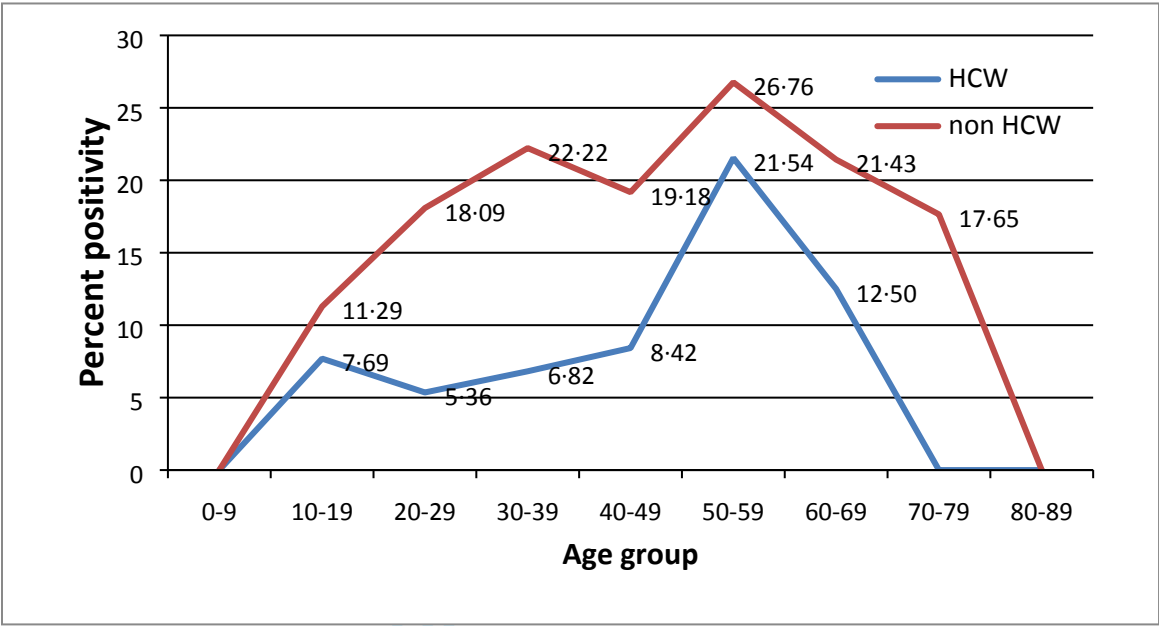


Figure 8: age group wise sero-positivity proportion among HCWs and non-HCWs among close contacts

# Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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			Page
Reporting Item			Number
<b>Title and abstract</b>			
Title	<a href="#">#1a</a>	Indicate the study's design with a commonly used term in the title or the abstract	1
Abstract	<a href="#">#1b</a>	Provide in the abstract an informative and balanced summary	3

1			of what was done and what was found	
2				
3				
4	<b>Introduction</b>			
5				
6				
7	Background /	<a href="#">#2</a>	Explain the scientific background and rationale for the	5
8	rationale		investigation being reported	
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11				
12	Objectives	<a href="#">#3</a>	State specific objectives, including any prespecified	6
13			hypotheses	
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16				
17	<b>Methods</b>			
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19				
20	Study design	<a href="#">#4</a>	Present key elements of study design early in the paper	7
21				
22				
23	Setting	<a href="#">#5</a>	Describe the setting, locations, and relevant dates, including	7-8
24			periods of recruitment, exposure, follow-up, and data collection	
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29	Eligibility criteria	<a href="#">#6a</a>	Give the eligibility criteria, and the sources and methods of	8
30			selection of participants.	
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34		<a href="#">#7</a>	Clearly define all outcomes, exposures, predictors, potential	8
35			confounders, and effect modifiers. Give diagnostic criteria, if	
36			applicable	
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42	Data sources /	<a href="#">#8</a>	For each variable of interest give sources of data and details of	9
43	measurement		methods of assessment (measurement). Describe	
44			comparability of assessment methods if there is more than one	
45			group. Give information separately for for exposed and	
46			unexposed groups if applicable.	
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54	Bias	<a href="#">#9</a>	Describe any efforts to address potential sources of bias	8
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57	Study size	<a href="#">#10</a>	Explain how the study size was arrived at	n/a
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Quantitative variables	<a href="#">#11</a>	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	n/a
Statistical methods	<a href="#">#12a</a>	Describe all statistical methods, including those used to control for confounding	25
Statistical methods	<a href="#">#12b</a>	Describe any methods used to examine subgroups and interactions	n/a
Statistical methods	<a href="#">#12c</a>	Explain how missing data were addressed	n/a
Statistical methods	<a href="#">#12d</a>	If applicable, describe analytical methods taking account of sampling strategy	n/a
Statistical methods	<a href="#">#12e</a>	Describe any sensitivity analyses	n/a
<b>Results</b>			
Participants	<a href="#">#13a</a>	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for for exposed and unexposed groups if applicable.	12
Participants	<a href="#">#13b</a>	Give reasons for non-participation at each stage	n/a
Participants	<a href="#">#13c</a>	Consider use of a flow diagram	n/a
Descriptive data	<a href="#">#14a</a>	Give characteristics of study participants (eg demographic,	12

1		clinical, social) and information on exposures and potential	
2		confounders. Give information separately for exposed and	
3		unexposed groups if applicable.	
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8	Descriptive data	<a href="#">#14b</a> Indicate number of participants with missing data for each	12
9		variable of interest	
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13	Outcome data	<a href="#">#15</a> Report numbers of outcome events or summary measures.	12
14		Give information separately for exposed and unexposed	
15		groups if applicable.	
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21	Main results	<a href="#">#16a</a> Give unadjusted estimates and, if applicable, confounder-	n/a
22		adjusted estimates and their precision (eg, 95% confidence	
23		interval). Make clear which confounders were adjusted for and	
24		why they were included	
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31	Main results	<a href="#">#16b</a> Report category boundaries when continuous variables were	25
32		categorized	
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36	Main results	<a href="#">#16c</a> If relevant, consider translating estimates of relative risk into	n/a
37		absolute risk for a meaningful time period	
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41	Other analyses	<a href="#">#17</a> Report other analyses done—e.g., analyses of subgroups and	n/a
42		interactions, and sensitivity analyses	
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47	Discussion		
48			
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50	Key results	<a href="#">#18</a> Summarise key results with reference to study objectives	19
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53	Limitations	<a href="#">#19</a> Discuss limitations of the study, taking into account sources of	13-14
54		potential bias or imprecision. Discuss both direction and	
55		magnitude of any potential bias.	
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1	Interpretation	<a href="#">#20</a>	Give a cautious overall interpretation considering objectives,	21
2			limitations, multiplicity of analyses, results from similar studies,	
3			and other relevant evidence.	
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8	Generalisability	<a href="#">#21</a>	Discuss the generalisability (external validity) of the study	21
9			results	
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14	<b>Other Information</b>			
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16				
17	Funding	<a href="#">#22</a>	Give the source of funding and the role of the funders for the	23
18			present study and, if applicable, for the original study on which	
19			the present article is based	
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27 made by the [EQUATOR Network](#) in collaboration with [Penelope.ai](#)  
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# BMJ Open

## ASSESSING SEROPOSITIVITY FOR IgG ANTIBODIES AGAINST SARS-CoV2 IN AHMEDABAD CITY OF INDIA

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-044101.R1
Article Type:	Original research
Date Submitted by the Author:	19-Nov-2020
Complete List of Authors:	Prakash, Om; Ahmedabad Municipal Corporation Solanki, Bhavin; Ahmedabad Municipal Corporation, Health Department SHETH, JAY; AMC MET Medical College, Community Medicine Joshi, Bhavin; Ahmedabad Municipal Corporation Kadam, Mina; AMC MET Medical College, Department of Microbiology Vyas, Sheetal; AMC MET Medical College, Department of Community Medicine Shukla, Aparajita; Smt NHL Municipal Medical College, Department of Community Medicine Tiwari, Hemant; Smt NHL Municipal Medical College, Department of Community Medicine Rathod, Sanjay; AMC MET Medical College, Department of Microbiology Rajput, Anil; AMC MET Medical College, Department of Microbiology Trivedi, Toral; AMC MET Medical College, Department of Microbiology Ramanuj, Vaibhav; Smt NHL Municipal Medical College, Department of Community Medicine Solanki, Anand; AMC MET Medical College, Department of Community Medicine
<b>Primary Subject Heading</b>:	Public health
Secondary Subject Heading:	Epidemiology, Immunology (including allergy), Global health, Infectious diseases
Keywords:	COVID-19, IMMUNOLOGY, VIROLOGY

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**TITLE: ASSESSING SEROPOSITIVITY FOR IgG ANTIBODIES AGAINST  
SARS-CoV2 IN AHMEDABAD CITY OF INDIA**

**Authors:**

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Word Count

Abstract: 277

Article: 3295

# **Title:** ASSESSING SEROPOSITIVITY FOR IgG ANTIBODIES AGAINST SARS-CoV2 IN AHMEDABAD CITY OF INDIA

## **ABSTRACT**

Objectives: To study the percentage sero-positivity for SARS-CoV2 to understand the pandemic status and predict the future situations in Ahmedabad

Study Design: Cross Sectional study

Settings: Field area of Ahmedabad Municipal Corporation

Participants: more than 30,000 individuals irrespective of their age, sex, acute/past covid19 infection participated in the sero-survey which covered all the 75 Urban Primary Health Centres (UPHCs) across 48 wards and 7 zones of the city. Study also involved Health Care Workers (HCW) from Covid/Non-Covid hospitals.

Interventions: Seropositivity of IgG antibodies against SARS-CoV2 was measured as a mark of Covid19 infection

Primary and Secondary Outcomes: Seropositivity was used to calculate cumulative incidence.

Correlation of seropositivity with available demographic detail was used for valid and precise assessment of the pandemic situation.

Results: From 30054 samples, the results were available for 29891 samples and the crude seropositivity is 17.61%. For all the various age groups, the seropositivity calculated between 15%-20%. The difference in seropositivity for both the sex group is statistically not significant. The sero-positivity is significantly lower (13.64%) for HCWs as compared to non-HCWs (18.71%). Seropositivity shows increasing trend with time. Zone with maximum initial cases have

high positivity as compared to other zones. UPHCs with recent rise in cases are leading in seropositivity as compared to earlier and widely affected UPHCs.

Conclusions: The results of sero-surveillance suggest that the population of Ahmedabad is still largely susceptible. People still need to follow preventive measures to protect themselves till an effective vaccine is available to the people at large. The data indicate the possibility of vanishing immunity over time and need further research to cross verify with scientific evidences.

Key words: SARS-COV2; Covid-19, Sero-surveillance; IgG antibodies, Immunity

**Strengths and Limitations of the study**

- Large representative sample of more than 30,000 people, including Health Care Workers
- Interesting scientific findings on the seropositivity for IgG antibodies against SARS-COV2
- Seropositivity also compared with reported cases
- Limited demographic detail available to check correlation with seropositivity

## Introduction:

A new respiratory virus causing severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) was first reported from China in December 2019 and soon spread throughout the world.<sup>1,2</sup> The world health organization (WHO) declared it as Pandemic and named the disease caused by this virus as COVID-19.<sup>3</sup> Being a newly identified virus, the scientific community is largely unaware of the natural history and the immune response developed after the Covid19 infection.<sup>4</sup> Since the virus is novel in origin, the initial seropositivity in the population is nil. So, a population based sero-survey can help in estimating cumulative incidence of a novel infection as well as extent of the infection in the community.<sup>5</sup> WHO also recommends monitoring of sero-prevalence over time for anticipating disease dynamics and planning an adequate public health response.<sup>6</sup> The sample size in such sample survey should be large enough to get reliable parameters sufficient enough to draw conclusions and future public health actions.<sup>7</sup>

Serological tests and the sero-epidemiology greatly helps in understanding the disease transmission, population susceptibility as well as the public health measures to be followed.<sup>8</sup> Since, the test for Covid19 infection turns out to be positive even in an asymptomatic patient, restricting the serological testing only to symptomatic individual will not give a real picture.<sup>9</sup> On the other hand a field level population based testing will give a better assessment of disease situation and the specific immunity following its infection. While the positive results indicate what proportion of the testing population has developed complete or partial immunity, those with negative result gives hint about the proportion of susceptible population.

India being the second most populous country with high population density is at high risk from covid19 pandemic. Ahmedabad city was among the first few cities severely affected by the spread of the pandemic. Ahmedabad city of India, having 7 million population, was one of the earliest cities to witness the high case load in the initial months of the pandemic in India. Ahmedabad had approximately 16360 reported Covid-19 cases and 1184 reported Covid-19 deaths before start of this study. A sero-surveillance study by the ICMR in the containment zones of the city carried out on 28-29 May 2020 had reported 55% seroprevalence, the highest among all the containment zones from all the major cities of India.<sup>10</sup> Central zone of the city was one of the highly affected area which was kept under area containment for more than a month from 26<sup>th</sup> April 2020. With such high number of cases and transmission, Ahmedabad was ideally suited to study the percentage sero-positivity in general population to help understand the pandemic status and predict the future situations.

**Aim:**

- To analyse and study the available data related to Covid-19 sero-positivity in Ahmedabad City

**Objectives:**

- To calculate the sero-prevalence of Ig-G antibodies to COVID-19 in the general population in Ahmedabad
- To correlate the sero-positivity with various factors for better understanding of the pandemic situation
- To identify the immunity status for valid & precise predictions for the future.

## Methodology

Looking to monitor the pandemic, understand its present situation and to take appropriate corrective public health measures, the Indian Council of Medical Research (ICMR) issued directives to all the state governments to carry out IgG Elisa test for sero-surveys along with ILI & SARI Surveillance.<sup>11</sup> The primary purpose of this was to understand the proportion of population exposed to SARS-CoV-2 including asymptomatic individuals. The Health department of the local municipality of Ahmedabad – Ahmedabad Municipal Corporation (AMC), from the state of Gujarat, INDIA, carried out a large-scale population based serological survey for IgG antibodies against SARS-CoV-2. This was one of the earliest primary situational analysis of the immune status against the SARS CoV2 infection from India.

Ahmedabad city is divided into 48 wards distributed across 7 zones. There are 75 UPHCs which cater the primary health care services to the local population. To get the real status of immunity, authorities of AMC preferred a field level serological study over case load or case density related sampling. At the UPHC, a trained laboratory technician is available along with necessary basic laboratory support for handling the sample, and so, the sample collection in the field area were managed by the staff posted at the UPHCs. For enrollment in the study, convenience sampling was followed at the level of UPHCs. The field level HCWs facilitated the enrollment of the willing individuals for the purpose of the study. An effort was made to cover a wide variety of people of different age groups from both the gender and falling into various categories without any exception. Thus, the inclusion criteria allowed inclusion of all irrespective of age, sex, acute/past covid19 infection. Exclusion criteria included refusal to give informed verbal consent or any contraindication to venipuncture.

Ahmedabad Municipal Corporation manages 1 dedicated Covid19 hospital which is attached with a medical college. There are 2 other non-Covid municipal general hospitals which are attached with medical colleges. As many of the health workers working at these hospitals have developed covid19 infection during the first 3 months of the pandemic, the authorities were concerned about their immune status and invited willing HCWs to enroll from the 3 hospitals under their administrative control to participate. So, apart from the 75 UPHCs, enrollment of the HCWs was also carried out at these 3 hospitals for the purpose of antibody testing.

An informed verbal consent was taken from all the participants before enrollment. Strict confidentiality was ensured at all the levels. Looking to the sample handling capacity at the UPHC and the testing capacity of the laboratories, the study continued over a period of approximately 3 weeks (from 16<sup>th</sup> June to 7<sup>th</sup> July). For the purpose of quality testing and reliability of results, only medical college laboratories were considered. Accredited private laboratories with all necessary equipment and facilities, which routinely undergo external quality assurance were also considered for timely results of the large number of samples.

“Covid Kavach” (Anti-SARS CoV-2 IgG Antibody Detection capture ELISA) kits developed and manufactured by Zydus Diagnostics, validated by National Institute of Virology, Pune, India and Approved for use by the Indian Council of Medical Research (ICMR) was used for the purpose of this study after due approval. As per the validation reports, the kits have a sensitivity of 92.37% and a specificity of 97.9%.<sup>12</sup> So, with very high level of sensitivity and specificity it may be noted that the results received through this testing kit is highly reliable and the kit is permitted for use in

sero-surveillance of SARS-CoV-2. The manufacturer reported no cross-reactivity with other viruses in the serum from real-time RTqPCR confirmed patients of various other infections. Testing procedures were followed as per the manufacturer's instructions. For each plate, samples with optical density (OD) value more than the cut-off value and positive/negative (P/N) ratio more than 1.5 were considered as positive. Samples with OD value of 10 per cent  $\pm$  ranges of the cut-off were considered to be indeterminate. The P/N ratio was defined as the ratio of average OD value of the positive control divided by the average OD of the negative control. The cut-off OD value was calculated as the average OD value of negative control +0.2.

AMC had collected the data of the enrolled participants in a simple brief format with minimum basic primary demographic information. An in-depth analysis of available data was crucial for valid and precise estimation of immunity status and for guiding the authorities for taking appropriate public health measures in a timely manner. So, the faculty members involved in corona control room activities, field monitoring & supervision as well as antibody testing were invited to critically analyse the available data and draw scientific conclusion on the level of seropositivity. Approval to study and analyze the available data was granted by the Institutional Review Board. We tried to find out the crude positivity as an indicator for the level of cumulative incidence and compared it with various other factors. We shared the results with the concerned authorities to take appropriate public health measures for larger benefit of the population. We herewith share the results for the detailed insight by the scientific community.

Patient and Public Involvement: patients were not directly involved in the planning or implementation of the sero-surveillance. However, these are carried out by the administrators of

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the local self-governance (Municipal Corporation) and was carried out for their benefit and determining their immunity status. The results were declared to the local media through press conference and were widely publicized on social media through executive summary and published by local daily news-papers as well.

For peer review only

## Results

A total of 30054 blood samples were collected for the purpose of this study, of which 1511 samples were collected from the 3 hospitals while the remaining 28543 samples were collected from the 75 UPHCs. A total of 163 samples were rejected for various reasons. Results were available for the remaining 29891 samples (Table-1). A total of 24197 tests were Negative while 431 test results were reported as indeterminate. Thus, a total of 5263 results were positive for the specific antibodies against Covid19 giving an overall crude positivity of about 17.61% (Figure-1)

There were 16135 males and 13919 females enrolled in the study and results were available for 29891 individuals. A total of 2774 from 16044 males were tested positive giving the positivity rate of 17.29%. A total of 2489 out of 13847 females were tested positive giving the positivity rate of 17.98%. Thus, the percentage positivity is slightly higher among females as compared to the males but the difference is statistically not significant ( $P > 0.05$ ).

The age wise analysis shows that the age of the sample population ranges from <1 year to 100 years with an average of 39.10 years with a standard deviation of 14.54 years. Among the sample, the mean age of females is 37.70 years with a Standard Deviation of 13.93 years, whereas the mean age of males is 40.25 years with a standard deviation of 14.88 years. Considering the sero-positive, the mean age for females is 38.69 years with a SD of 14.16 years where as that of male is 41.41 years with a SD of 14.51 years.

The age group wise analysis of total tests and positive tests when compared to calculate percent positivity (Figure-2) shows that the positivity in various age-groups is between 15-20 percent.

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Considering the gender variation also for different age groups (Figure-3), the percent positivity for both the genders is seen between 15 to 20 percent for most of the age-groups. The age-group wise distribution is statistically significant.

UPHC wise positivity was compared for the first 10 UPHCs with highest positivity (Table-2). The comparison of zone wise positivity with cases (Table-3) shows that the zone most affected by covid19 i.e. Central zone with maximum initial cases had the highest positivity of 28.54%. Thus, the positive antibody status correlates with the documented evidence of high case-load particularly in the first 2 months of the pandemic in Ahmedabad.

Our study included HCWs from field area as well as Covid/Non-Covid hospitals. With 888 sero-positives out of 6509 HCWs, the seropositivity among HCWs is 13.64%. On the other hand, seropositivity among non-HCWs was 18.71%. Seropositivity among non-HCW is significantly higher as compared to the HCWs.

We also tried to compare positivity with days of our study period, which showed wide variation. To remove the effect of daily variation we tried to study the linear trend (Figure-4). We tried to calculate the weekly seropositivity for the 3-week study duration (Table-1), which shows a seropositivity of 13.83%, 20.20% and 22.33% respectively. Zone wise seropositivity & its comparison with cases per 10000 population (Figure-5) and case density (Figure-6) shows that the positivity correlates with the cases in most zones but is strikingly low in Central zone.

## Discussion:

The present study is one of the biggest studies conducted in the world in terms of sample population ratio to find out sero positivity in general population. This study has 4770 samples per million population in comparison to Spanish study where the sample population ratio was 1302 samples per million population and the US study where the sample population ratio was 255 samples per million population.<sup>13,14</sup> With 17.61% seropositivity our results are consistent with other studies showing that even in the areas highly affected by SARS CoV2 during this pandemic, have shown very low level of seropositivity.<sup>8</sup> Antibodies do take some time to develop after an infection, approximately 1 to 3 weeks, with an average of 2 weeks (14 days).<sup>15,16</sup> So, we can say that the rate of antibody positivity reflects the case scenario about 14 days prior to the study. A national seroprevalence study from India, completed a few days before the present study, documented the national seroprevalence of just 0.73% [95% Confidence Interval (CI) 0.34-1.13]. The vast difference reflected in our study, justify our statement for the situation in Ahmedabad during the initial 3 months of the pandemic.<sup>17</sup> On the other hand, the study by ICMR found 55% seroprevalence in the containment zone.<sup>10</sup>

The difference in the positivity for both the sex group is statistically not significant and this finding is similar to other studies from Spain & USA where the studies have found no significant difference between the genders for covid19 antibodies.<sup>7,8,18</sup> Comparatively less samples with wide variation in positivity at the extremes of age group i.e. 0-9 years and 90-99 years for both the gender groups may be the reason for having a statistically significance difference for the age group wise seropositivity. Majority of the HCWs in the study were from the field area – working at the UPHCs. A statistically significant low sero-positivity among HCW as compared to non-HCW

indicate that they are better protected as compared to general population, particularly when the epidemic has progressed to infect a large number of people from general population.

Our results also show that the UPHCs with high number of cases have higher positivity. However, UPHCs with high number of cases in the early phase of the pandemic have low seropositivity as compared to the UPHCs with higher number of cases during the later part of the pandemic (with more cases in the recent past). In Ahmedabad, the initial cases were more from the central zone followed by south zone. Then, gradually due to extensive containment measures the cases from Central zone reduced and cases started appearing more from the East and North zones. It can be seen that the highest seropositivity was found in the individuals from East Zone and North Zone. This is on a slightly higher side than that from the UPHCs of the Central Zone, which had the overall highest number of cases. Strikingly the worst and first affected UPHCs of the Central zone have lesser positivity. The first 8 UPHCs with the highest positivity are from North Zone & East Zone (Having higher cases in the later part of the pandemic period so far) than that of Central zone (having maximum population density and highest cases in the very first phase of pandemic period). This might be pointing towards the fact that the immunity developed after a successful recovery from Covid19 infection may not be lasting enough. Although the same is also reported by other studies, we need more studies and longer follow up to cross verify this aspect and to bring out the scientific fact related with the post-covid immunity.<sup>19-21</sup>

Day to day variation in positivity may be dependent on the proportion of different population groups (e.g. cases, contacts, super spreaders, symptomatic, asymptomatic, HCWs etc.) covered on that day. However, the trend suggest that the positivity slowly increases with time. The same is

the conclusion from the week-wise positivity calculated for the 3 weeks study duration. Both of these findings show that the positivity increase with time & thus correlates with increasing number of cumulative cases.

When we compare cases per million population with sero positivity (Table-3, Figure-5) it was found that most affected central zone with highest cases (5743 per million population) also had highest seropositivity of 28.54%. However, the areas of recent infection, like North Zone and East zone, have higher sero positivity (27.41% & 23.44% respectively) despite having lower cases (2649 & 1777 cases per million population respectively) than the South Zone. The comparative low positivity as compared to high case per million population may be largely due to the sampling method followed, which was independent of the cases from that zone. We also tried to compare the case density (cases per square Km) with sero positivity (Table-3, Figure-6). Here also the findings suggest that the first and worst affected central zone with highest case density had highest seropositivity. Areas of recent infection like North Zone and East zone have higher sero positivity of 27.41% and 23.44% respectively (much higher than that of south zone, i.e. 16.81%) despite having case density comparative with that of south zone (44.5 and 34.6 cases per square Km respectively, as against 37.0 cases per square Km in South Zone). Both this comparison indicates the possibility of vanishing positivity with the passing of time. However, we need more in-depth scientific research to find out the reason behind this paradox.

Looking at the positivity rate at the Covid/Non-Covid hospitals, it is observed that the highest positivity (12.84%) is seen at the SVP hospital which is a dedicated Covid hospital right from the start of the corona pandemic in the city. The overall positivity of 12.84% is still lower than that of

the crude positivity of the general population i.e. 17.61%. This may be due to the fact that all the HCWs in a dedicated Covid hospital are very well protected with PPE & other safety measures.

LG hospital is a non-Covid hospital attached with another medical college run by the AMC. The positivity rate at LG is also quite similar to SVP hospital with 12.80% positivity. Although LG hospital is not a dedicated covid hospital, still the positivity rate here is quite similar to that of the covid hospital. Inspite of being a non-Covid hospital, there were multiple occasions in the previous 2 months when large number of HCWs turned out to be positive & many HCWs were isolated/quarantined. A lot of patients from the containment zone reported here for non-Covid complains but were tested & reported positive. The authorities were even forced to close down the hospital once for about 7 days for improving the sanitization measures and improving the implementation of various SOPs. As compared to the above two hospitals, the other non-Covid hospital (SCL Hospital) did not have many Covid cases and the low positivity (3.09%) at this hospital also correlate with the data.

### **Limitation of our study:**

The study followed convenience sampling at the level of UPHC. Some of the collected data (e.g. symptomatology of the enrolled individual) was not completely available for data analysis.

### **Conclusion:**

This study to assess the seropositivity during the Covid19 pandemic from Ahmedabad, India is one of the first few population-based study from India with a large sample size and a very high sample-population ratio. As of June 2020, the level of Covid-19 seropositivity in Ahmedabad city, India is 17.61%. In view of these findings with the absence of an evidence of lifelong immunity after Covid-19 infection, it can be concluded that the population of Ahmedabad is still largely susceptible. As of now, we cannot rely on this level of immunity to protect and the preventive measures need to be strongly relied upon till an effective vaccine is provided to the people at large. There is no gender difference in sero-positivity but the seropositivity is significantly associated with the risk of covid-19 infection in the area. Although the sero-positivity slowly increases with time there are also indications that these IgG may not be long lasting. Further in-depth scientific studies are required to give more insight for the future predictions.

**Author Contributions:**

Dr. Bhavin Solanki (BS) under the guidance of Dr. OM Prakash (OP) planned and carried out the sero-surveillance. Testing of the samples and reporting was managed by Dr. Sanjay Rathod (SR), Dr. Anil Rajput (AR) and Dr. Toral Trivedi (TT) under the leadership of Dr. Mina Kadam (MK). Data analysis was carried out by Dr. Jay Sheth (JS), Dr. Sheetal Vyas (SV), Dr. Aparajita Shukla (ASh), Dr. Bhavin Joshi (BJ), Dr. Vaibhav Ramanuj (VR) and Dr. Anand Solanki (ASo) while the statistical analysis was done by Dr. Hemant Tiwari (HT). Primary manuscript was prepared by Dr. Om Prakash (OP) and Dr. Jay Sheth (JS) and equally contributed by all the other co-authors. All authors contributed to the interpretation of data and approved the final manuscript after critical review.

**Declaration of interests:**

We declare no competing interests.

**Funding:** Health Department of Ahmedabad Municipal Corporation carried out the study as part of Covid19 pandemic response. There is no external funding

**Data sharing:**

Our data are accessible to researchers upon reasonable request for data sharing to the corresponding author.

**Ethical permission:**

We have received permission for the study from the Institutional Review Board (IRB) of the AMC MET Medical College, Ahmedabad.

### **Acknowledgments:**

We are extremely thankful to respected Dr. Rajiv Kumar Gupta, IAS (Additional Chief Secretary, Government of Gujarat) and Mr. Mukesh Kumar, IAS (Municipal Commissioner, Ahmedabad) for their whole hearted support. This study would not have been possible without the financial support from the authorities of Ahmedabad Municipal Corporation. We acknowledge the full support from the field level health care workers (Corona warriors) who put in great efforts to perform their duties as well as sample collection after informed verbal consent particularly in a Covid-19 pandemic situation. All the Zonal Deputy Health Officers, Deputy Health Officer (Epidemic), Assistant Health Officers and Medical officers of the Urban Primary Health Centers extended full support in conducting the sero-surveillance. We are thankful to all the medical and paramedical support staff posted at the laboratories for their contribution in timely testing such large number of samples with accuracy and quality. Finally, we are indebted to all the patients including health care workers whose willingness and support has generated the much-desired data for the study.

Table-1 Analysis of Covid19 sero-survey positivity

	Female			Male			Total			95% CI	P Value
	Results	Positive	% Positivity	Results	Positive	% Positivity	Results	Positive	% Positivity		
Gender	13847	2489	17.98	16044	2774	17.29	29891	5263	17.61	17.18-18.04	P>0.05
Age group											
0-9	39	3	7.69	53	14	26.42	92	17	18.48	11.15-27.93	P < 0.001
10-19	552	92	16.67	687	106	15.43	1239	198	15.98	14.05-18.13	
20-29	4013	684	17.04	3665	518	14.13	7678	1202	15.66	14.86-16.49	
30-39	3424	569	16.62	3910	636	16.27	7334	1205	16.43	15.60-17.30	
40-49	2837	528	18.61	3162	668	21.13	5999	1196	19.94	18.95-20.97	
50-59	1749	356	20.35	2608	499	19.13	4357	855	19.62	18.47-20.83	
60-69	889	189	21.26	1356	222	16.37	2245	411	18.31	16.76-19.96	
70-79	291	60	20.62	505	95	18.81	796	155	19.47	16.87-22.37	
80-89	49	8	16.33	93	14	15.05	142	22	15.49	9.97-22.51	
90-99	4	0	0	5	2	40.00	9	2	22.22	2.81-60.01	
Zone											
CZ	1876	567	30.22	1897	510	26.88	3773	1077	28.54	27.13-30.01	P < 0.001
NZ	2222	579	26.06	2576	736	28.57	4798	1315	27.41	26.16-28.69	
EZ	1630	378	23.19	2039	482	23.64	3669	860	23.44	22.10-24.84	
SZ	1564	278	17.77	1649	262	15.89	3213	540	16.81	15.55-18.14	
SWZ	1145	167	14.59	1838	235	12.79	2983	402	13.48	12.30-14.75	
WZ	3193	351	10.99	3111	310	9.96	6304	661	10.49	09.75-11.27	
NWZ	1359	76	5.59	2303	160	6.95	3662	236	6.44	05.69-07.29	
Hospital											
SVP (Covid)	339	37	10.91	331	49	14.80	670	86	12.84	10.51-15.58	P < 0.001
LG	418	55	13.16	207	25	12.08	625	80	12.80	10.41-15.65	
SCL	101	1	0.99	93	5	5.38	194	6	3.09	01.14-06.61	
Study week											
Week1	6535	942	14.41	6654	882	13.26	13189	1824	13.83	13.25-14.43	P < 0.001
Week2	5840	1208	20.68	7826	1553	19.84	13666	2761	20.20	19.54-20.88	
Week3	1472	339	23.03	1564	339	21.68	3036	678	22.33	20.89-23.85	
Category											
NonHCW	10114	2003	19.80	13268	2372	17.88	23382	4375	18.71	18.22-19.22	P < 0.001
HCW	3733	486	13.02	2776	402	14.48	6509	888	13.64	12.83-14.50	

Table-2 Top ten UPHC with highest seropositivity results

UPHC of AMC	Total	Results	Positive	Positivity
MEGHANINAGAR (NZ)	119	119	52	43.70
GOMTIPUR (EZ)	308	300	118	39.33
RAJPUR (EZ)	383	383	138	36.03
NARODA ROAD (NZ)	170	170	61	35.88
INDIACOLONY (NZ)	806	802	280	34.91
BAPUNAGAR (NZ)	316	316	110	34.81
KUBERNAGAR (NZ)	480	477	166	34.80
SARASPUR-RAKHIAL (NZ)	401	401	127	31.67
JAMALPUR (CZ)	429	428	135	31.54
SHAHPUR (CZ)	394	394	124	31.47

Table-3 Zone wise comparison of seropositivity and Covid19 cases

Zone	Population	Area (Sq Km)	Cases (As on June 15, 2020)	Case Density (Cases /Sq Km)	Cases per 10000 population	Results	positive	Percent positivity
CZ	683089	18.33	3923	214.0	57.43	3773	1077	28.54
SZ	1081996	88.65	3276	37.0	30.28	3213	540	16.81
NZ	1090409	64.92	2889	44.5	26.49	4798	1315	27.41
EZ	1425254	73.2	2532	34.6	17.77	3669	860	23.44
WZ	1302500	65.68	2357	35.9	18.10	6304	661	10.49
SWZ	524970	61.86	871	14.1	16.59	2983	402	13.48
NWZ	807539	91.36	772	8.5	9.56	3662	236	6.44

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3 Figure-1 Result of Sero-surveillance  
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5 Figure-2 Age group wise Sero-positivity  
6  
7 Figure-3 Age group & sex wise Sero-positivity  
8  
9 Figure-4 Day wise Sero-Positivity  
10  
11 Figure-5 Comparison of seroprevalence with zone wise cases  
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13 Figure-6 Comparison of Seroprevalence with case density  
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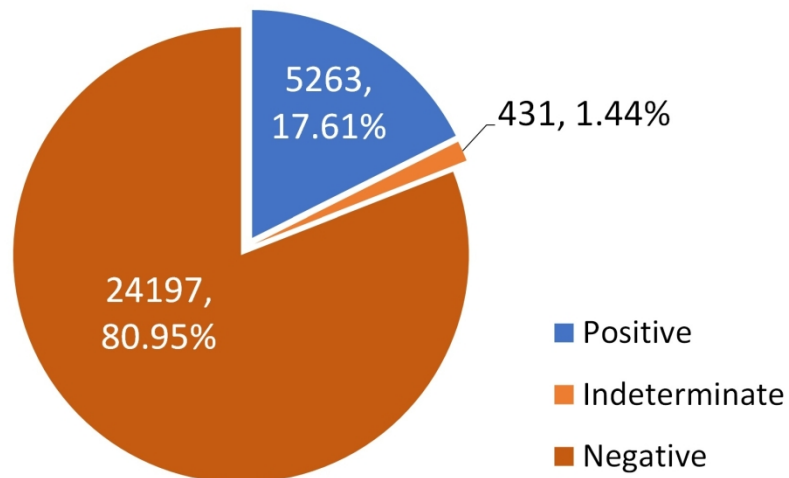
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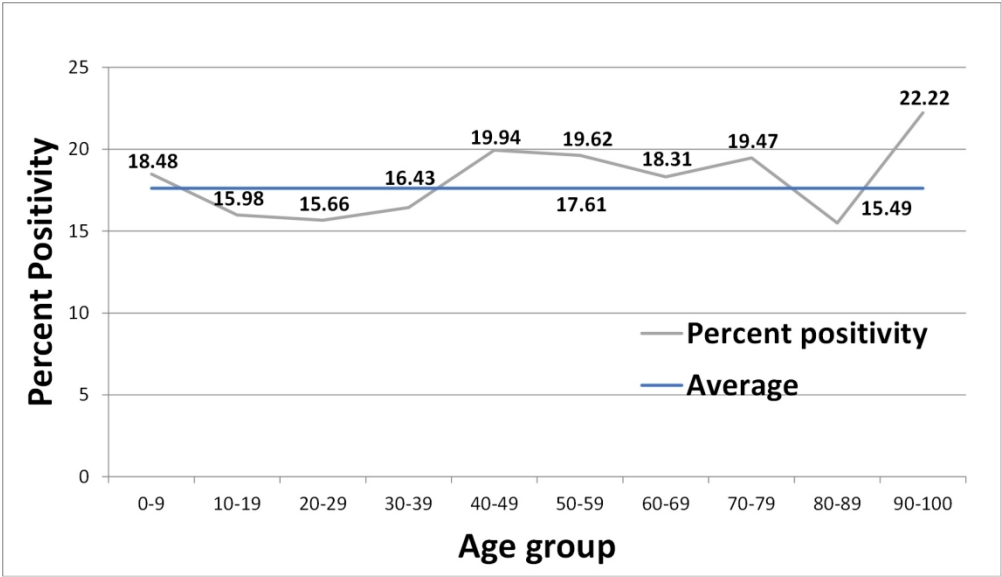
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## Result of Sero-surveillance



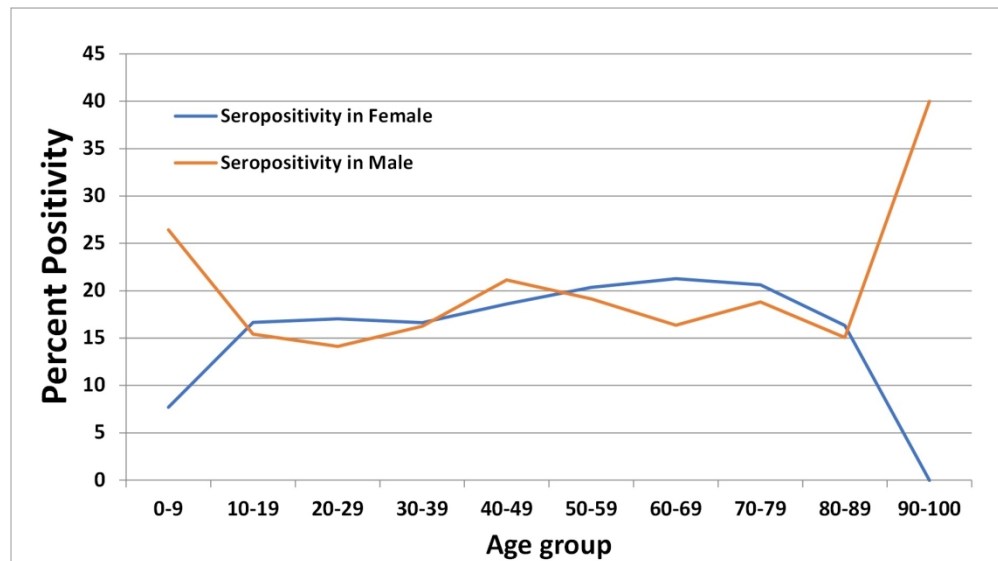
Result of Sero-surveillance

175x109mm (300 x 300 DPI)



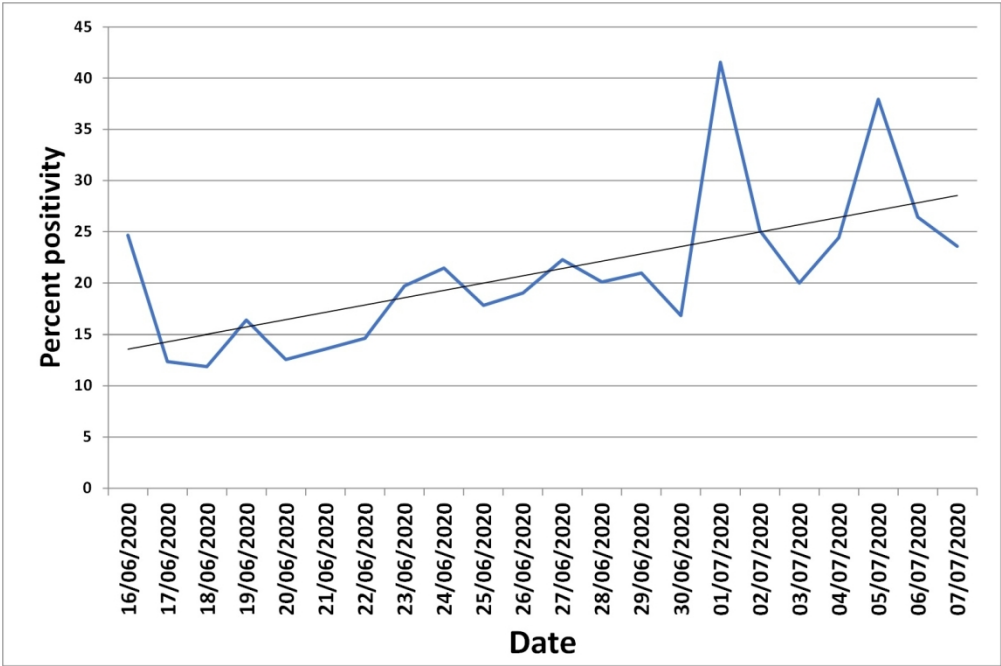
Age group wise Sero-positivity

156x90mm (300 x 300 DPI)



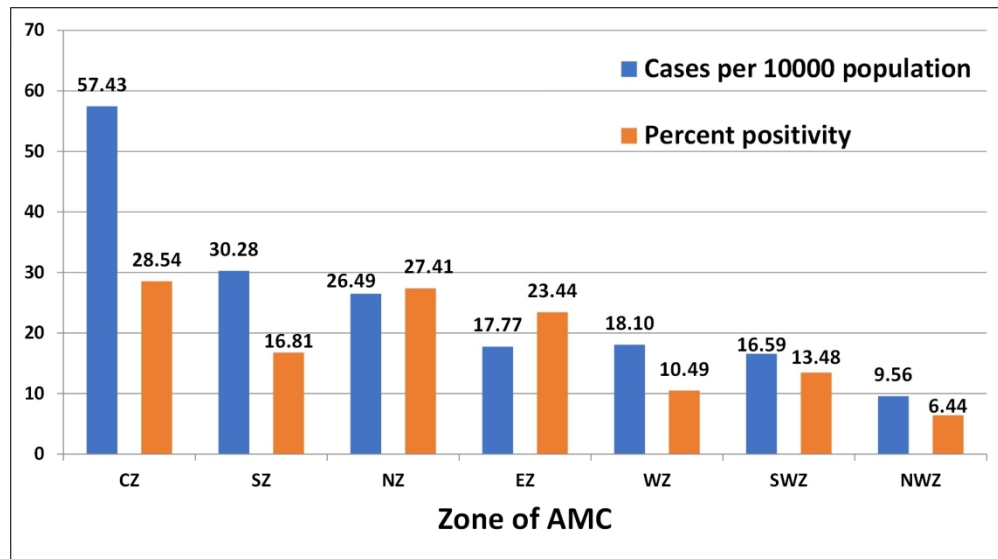
Age group & sex wise Sero-positivity

161x90mm (300 x 300 DPI)



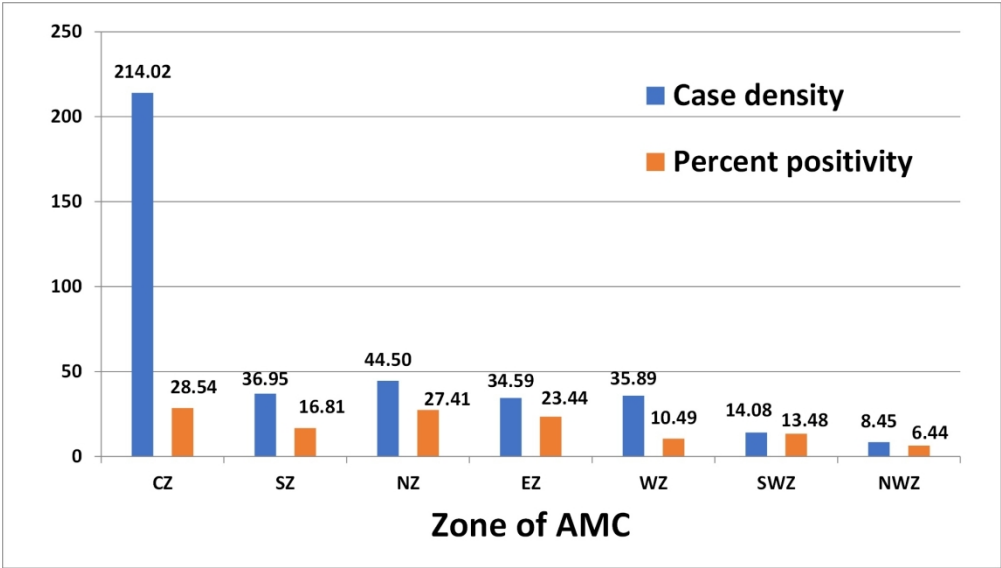
Day wise Sero-Positivity

135x90mm (300 x 300 DPI)



Comparison of seroprevalence with zonewise cases

161x90mm (300 x 300 DPI)



Comparison of Seroprevalence with case density

176x99mm (300 x 300 DPI)

# Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

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			Page
Reporting Item			Number
<b>Title and abstract</b>			
Title	<a href="#">#1a</a>	Indicate the study's design with a commonly used term in the title or the abstract	1

1	Abstract	<a href="#">#1b</a>	Provide in the abstract an informative and balanced summary	3
2			of what was done and what was found	
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5				
6	<b>Introduction</b>			
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10	Background /	<a href="#">#2</a>	Explain the scientific background and rationale for the	5
11	rationale		investigation being reported	
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15	Objectives	<a href="#">#3</a>	State specific objectives, including any prespecified	6
16			hypotheses	
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20	<b>Methods</b>			
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23	Study design	<a href="#">#4</a>	Present key elements of study design early in the paper	7
24				
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26	Setting	<a href="#">#5</a>	Describe the setting, locations, and relevant dates, including	7-9
27			periods of recruitment, exposure, follow-up, and data	
28			collection	
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34	Eligibility criteria	<a href="#">#6a</a>	Give the eligibility criteria, and the sources and methods of	7
35			selection of participants.	
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39		<a href="#">#7</a>	Clearly define all outcomes, exposures, predictors, potential	8-9
40			confounders, and effect modifiers. Give diagnostic criteria, if	
41			applicable	
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47	Data sources /	<a href="#">#8</a>	For each variable of interest give sources of data and details	8-9
48	measurement		of methods of assessment (measurement). Describe	
49			comparability of assessment methods if there is more than	
50			one group. Give information separately for for exposed and	
51			unexposed groups if applicable.	
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Bias	<a href="#">#9</a>	Describe any efforts to address potential sources of bias	8
Study size	<a href="#">#10</a>	Explain how the study size was arrived at	n/a
Quantitative variables	<a href="#">#11</a>	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	n/a
Statistical methods	<a href="#">#12a</a>	Describe all statistical methods, including those used to control for confounding	20
Statistical methods	<a href="#">#12b</a>	Describe any methods used to examine subgroups and interactions	n/a
Statistical methods	<a href="#">#12c</a>	Explain how missing data were addressed	n/a
Statistical methods	<a href="#">#12d</a>	If applicable, describe analytical methods taking account of sampling strategy	n/a
Statistical methods	<a href="#">#12e</a>	Describe any sensitivity analyses	n/a
<b>Results</b>			
Participants	<a href="#">#13a</a>	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for exposed and unexposed groups if applicable.	11
Participants	<a href="#">#13b</a>	Give reasons for non-participation at each stage	n/a

Participants	<a href="#">#13c</a>	Consider use of a flow diagram	n/a
Descriptive data	<a href="#">#14a</a>	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	11
Descriptive data	<a href="#">#14b</a>	Indicate number of participants with missing data for each variable of interest	11
Outcome data	<a href="#">#15</a>	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	11-12
Main results	<a href="#">#16a</a>	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n/a
Main results	<a href="#">#16b</a>	Report category boundaries when continuous variables were categorized	20
Main results	<a href="#">#16c</a>	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	<a href="#">#17</a>	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	n/a
<b>Discussion</b>			
Key results	<a href="#">#18</a>	Summarise key results with reference to study objectives	17

Limitations	<a href="#">#19</a>	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	17
Interpretation	<a href="#">#20</a>	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13-16
Generalisability	<a href="#">#21</a>	Discuss the generalisability (external validity) of the study results	13-16
<b>Other Information</b>			
Funding	<a href="#">#22</a>	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

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# BMJ Open

## ASSESSING SEROPOSITIVITY FOR IgG ANTIBODIES AGAINST SARS-CoV2 IN AHMEDABAD CITY OF INDIA: A CROSS-SECTIONAL STUDY

Journal:	<i>BMJ Open</i>
Manuscript ID	bmjopen-2020-044101.R2
Article Type:	Original research
Date Submitted by the Author:	13-Dec-2020
Complete List of Authors:	Prakash, Om; Ahmedabad Municipal Corporation Solanki, Bhavin; Ahmedabad Municipal Corporation, Health Department SHETH, JAY; AMC MET Medical College, Community Medicine Joshi, Bhavin; Ahmedabad Municipal Corporation Kadam, Mina; AMC MET Medical College, Department of Microbiology Vyas, Sheetal; AMC MET Medical College, Department of Community Medicine Shukla, Aparajita; Smt NHL Municipal Medical College, Department of Community Medicine Tiwari, Hemant; Smt NHL Municipal Medical College, Department of Community Medicine Rathod, Sanjay; AMC MET Medical College, Department of Microbiology Rajput, Anil; AMC MET Medical College, Department of Microbiology Trivedi, Toral; AMC MET Medical College, Department of Microbiology Ramanuj, Vaibhav; Smt NHL Municipal Medical College, Department of Community Medicine Solanki, Anand; AMC MET Medical College, Department of Community Medicine
<b>Primary Subject Heading</b>:	Public health
Secondary Subject Heading:	Epidemiology, Global health, Infectious diseases, Immunology (including allergy)
Keywords:	COVID-19, IMMUNOLOGY, VIROLOGY

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**TITLE: ASSESSING SEROPOSITIVITY FOR IgG ANTIBODIES AGAINST SARS-CoV2 IN  
AHMEDABAD CITY OF INDIA: A CROSS-SECTIONAL STUDY**

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Word Count

Abstract: 277

Article: 3295

**Title:** ASSESSING SEROPOSITIVITY FOR IgG ANTIBODIES AGAINST SARS-CoV2 IN AHMEDABAD CITY OF INDIA: A CROSS-SECTIONAL STUDY

**ABSTRACT**

Objectives: To study the percentage sero-positivity for SARS-CoV2 to understand the pandemic status and predict the future situations in Ahmedabad

Study Design: Cross Sectional study

Settings: Field area of Ahmedabad Municipal Corporation

Participants: more than 30,000 individuals irrespective of their age, sex, acute/past covid19 infection participated in the sero-survey which covered all the 75 Urban Primary Health Centres (UPHCs) across 48 wards and 7 zones of the city. Study also involved Health Care Workers (HCW) from Covid/Non-Covid hospitals.

Interventions: Seropositivity of IgG antibodies against SARS-CoV2 was measured as a mark of Covid19 infection

Primary and Secondary Outcomes: Seropositivity was used to calculate cumulative incidence.

Correlation of seropositivity with available demographic detail was used for valid and precise assessment of the pandemic situation.

Results: From 30054 samples, the results were available for 29891 samples and the crude seropositivity is 17.61%. For all the various age groups, the seropositivity calculated between 15%-20%. The difference in seropositivity for both the sex group is statistically not significant. The sero-positivity is significantly lower (13.64%) for HCWs as compared to non-HCWs (18.71%). Seropositivity shows increasing trend with time. Zone with maximum initial cases have

high positivity as compared to other zones. UPHCs with recent rise in cases are leading in seropositivity as compared to earlier and widely affected UPHCs.

Conclusions: The results of sero-surveillance suggest that the population of Ahmedabad is still largely susceptible. People still need to follow preventive measures to protect themselves till an effective vaccine is available to the people at large. The data indicate the possibility of vanishing immunity over time and need further research to cross verify with scientific evidences.

Key words: SARS-COV2; Covid-19, Sero-surveillance; IgG antibodies, Immunity

**Strengths and Limitations of the study**

- Large representative sample of more than 30,000 people, including Health Care Workers
- Interesting scientific findings on the seropositivity for IgG antibodies against SARS-COV2
- Seropositivity also compared with reported cases
- Limited demographic detail available to check correlation with seropositivity

## Introduction:

A new respiratory virus causing severe acute respiratory syndrome corona virus 2 (SARS-CoV-2) was first reported from China in December 2019 and soon spread throughout the world.<sup>1,2</sup> The world health organization (WHO) declared it as Pandemic and named the disease caused by this virus as COVID-19.<sup>3</sup> Being a newly identified virus, the scientific community is largely unaware of the natural history and the immune response developed after the Covid19 infection.<sup>4</sup> Since the virus is novel in origin, the initial seropositivity in the population is nil. So, a population based sero-survey can help in estimating cumulative incidence of a novel infection as well as extent of the infection in the community.<sup>5</sup> WHO also recommends monitoring of sero-prevalence over time for anticipating disease dynamics and planning an adequate public health response.<sup>6</sup> The sample size in such sample survey should be large enough to get reliable parameters sufficient enough to draw conclusions and future public health actions.<sup>7</sup>

Serological tests and the sero-epidemiology greatly helps in understanding the disease transmission, population susceptibility as well as the public health measures to be followed.<sup>8</sup> Since, the test for Covid19 infection turns out to be positive even in an asymptomatic patient, restricting the serological testing only to symptomatic individual will not give a real picture.<sup>9</sup> On the other hand a field level population based testing will give a better assessment of disease situation and the specific immunity following its infection. While the positive results indicate what proportion of the testing population has developed complete or partial immunity, those with negative result gives hint about the proportion of susceptible population.

India being the second most populous country with high population density is at high risk from covid19 pandemic. Ahmedabad city was among the first few cities severely affected by the spread of the pandemic. Ahmedabad city of India, having 7 million population, was one of the earliest cities to witness the high case load in the initial months of the pandemic in India. Ahmedabad had approximately 16360 reported Covid-19 cases and 1184 reported Covid-19 deaths before start of this study. A sero-surveillance study by the ICMR in the containment zones of the city carried out on 28-29 May 2020 had reported 55% seroprevalence, the highest among all the containment zones from all the major cities of India.<sup>10</sup> Central zone of the city was one of the highly affected area which was kept under area containment for more than a month from 26<sup>th</sup> April 2020. With such high number of cases and transmission, Ahmedabad was ideally suited to study the percentage sero-positivity in general population to help understand the pandemic status and predict the future situations.

**Aim:**

- To analyse and study the available data related to Covid-19 sero-positivity in Ahmedabad City

**Objectives:**

- To calculate the sero-prevalence of Ig-G antibodies to COVID-19 in the general population in Ahmedabad
- To correlate the sero-positivity with various factors for better understanding of the pandemic situation
- To identify the immunity status for valid & precise predictions for the future.

## Methodology

Looking to monitor the pandemic, understand its present situation and to take appropriate corrective public health measures, the Indian Council of Medical Research (ICMR) issued directives to all the state governments to carry out IgG Elisa test for sero-surveys along with ILI & SARI Surveillance.<sup>11</sup> The primary purpose of this was to understand the proportion of population exposed to SARS-CoV-2 including asymptomatic individuals. The Health department of the local municipality of Ahmedabad – Ahmedabad Municipal Corporation (AMC), from the state of Gujarat, INDIA, carried out a large-scale population based serological survey for IgG antibodies against SARS-CoV-2. This was one of the earliest primary situational analysis of the immune status against the SARS CoV2 infection from India.

Ahmedabad city is divided into 48 wards distributed across 7 zones. There are 75 UPHCs which cater the primary health care services to the local population. To get the real status of immunity, authorities of AMC preferred a field level serological study over case load or case density related sampling. At the UPHC, a trained laboratory technician is available along with necessary basic laboratory support for handling the sample, and so, the sample collection in the field area were managed by the staff posted at the UPHCs. For enrollment in the study, convenience sampling was followed at the level of UPHCs. The field level HCWs facilitated the enrollment of the willing individuals for the purpose of the study. An effort was made to cover a wide variety of people of different age groups from both the gender and falling into various categories without any exception. Thus, the inclusion criteria allowed inclusion of all irrespective of age, sex, acute/past covid19 infection. Exclusion criteria included refusal to give informed verbal consent or any contraindication to venipuncture.

Ahmedabad Municipal Corporation manages 1 dedicated Covid19 hospital which is attached with a medical college. There are 2 other non-Covid municipal general hospitals which are attached with medical colleges. As many of the health workers working at these hospitals have developed covid19 infection during the first 3 months of the pandemic, the authorities were concerned about their immune status and invited willing HCWs to enroll from the 3 hospitals under their administrative control to participate. So, apart from the 75 UPHCs, enrollment of the HCWs was also carried out at these 3 hospitals for the purpose of antibody testing.

An informed verbal consent was taken from all the participants before enrollment. Strict confidentiality was ensured at all the levels. Looking to the sample handling capacity at the UPHC and the testing capacity of the laboratories, the study continued over a period of approximately 3 weeks (from 16<sup>th</sup> June to 7<sup>th</sup> July). For the purpose of quality testing and reliability of results, only medical college laboratories were considered. Accredited private laboratories with all necessary equipment and facilities, which routinely undergo external quality assurance were also considered for timely results of the large number of samples.

“Covid Kavach” (Anti-SARS CoV-2 IgG Antibody Detection capture ELISA) kits developed and manufactured by Zydus Diagnostics, validated by National Institute of Virology, Pune, India and Approved for use by the Indian Council of Medical Research (ICMR) was used for the purpose of this study after due approval. As per the validation reports, the kits have a sensitivity of 92.37% and a specificity of 97.9%.<sup>12</sup> So, with very high level of sensitivity and specificity it may be noted that the results received through this testing kit is highly reliable and the kit is permitted for use in

sero-surveillance of SARS-CoV-2. The manufacturer reported no cross-reactivity with other viruses in the serum from real-time RTqPCR confirmed patients of various other infections. Testing procedures were followed as per the manufacturer's instructions. For each plate, samples with optical density (OD) value more than the cut-off value and positive/negative (P/N) ratio more than 1.5 were considered as positive. Samples with OD value of 10 per cent  $\pm$  ranges of the cut-off were considered to be indeterminate. The P/N ratio was defined as the ratio of average OD value of the positive control divided by the average OD of the negative control. The cut-off OD value was calculated as the average OD value of negative control +0.2.

AMC had collected the data of the enrolled participants in a simple brief format with minimum basic primary demographic information. An in-depth analysis of available data was crucial for valid and precise estimation of immunity status and for guiding the authorities for taking appropriate public health measures in a timely manner. So, the faculty members involved in corona control room activities, field monitoring & supervision as well as antibody testing were invited to critically analyse the available data and draw scientific conclusion on the level of seropositivity. Approval to study and analyze the available data was granted by the Institutional Review Board. We tried to find out the crude positivity as an indicator for the level of cumulative incidence and compared it with various other factors. We shared the results with the concerned authorities to take appropriate public health measures for larger benefit of the population. We herewith share the results for the detailed insight by the scientific community.

Patient and Public Involvement: patients were not directly involved in the planning or implementation of the sero-surveillance. However, these are carried out by the administrators of

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the local self-governance (Municipal Corporation) and was carried out for their benefit and determining their immunity status. The results were declared to the local media through press conference and were widely publicized on social media through executive summary and published by local daily news-papers as well.

For peer review only

## Results

A total of 30054 blood samples were collected for the purpose of this study, of which 1511 samples were collected from the 3 hospitals while the remaining 28543 samples were collected from the 75 UPHCs. A total of 163 samples were rejected for various reasons. Results were available for the remaining 29891 samples (Table-1). A total of 24197 tests were Negative while 431 test results were reported as indeterminate. Thus, a total of 5263 results were positive for the specific antibodies against Covid19 giving an overall crude positivity of about 17.61% (Figure-1)

There were 16135 males and 13919 females enrolled in the study and results were available for 29891 individuals. A total of 2774 from 16044 males were tested positive giving the positivity rate of 17.29%. A total of 2489 out of 13847 females were tested positive giving the positivity rate of 17.98%. Thus, the percentage positivity is slightly higher among females as compared to the males but the difference is statistically not significant ( $P > 0.05$ ).

The age wise analysis shows that the age of the sample population ranges from <1 year to 100 years with an average of 39.10 years with a standard deviation of 14.54 years. Among the sample, the mean age of females is 37.70 years with a Standard Deviation of 13.93 years, whereas the mean age of males is 40.25 years with a standard deviation of 14.88 years. Considering the sero-positive, the mean age for females is 38.69 years with a SD of 14.16 years where as that of male is 41.41 years with a SD of 14.51 years.

The age group wise analysis of total tests and positive tests when compared to calculate percent positivity (Figure-2) shows that the positivity in various age-groups is between 15-20 percent.

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Considering the gender variation also for different age groups (Figure-3), the percent positivity for both the genders is seen between 15 to 20 percent for most of the age-groups. The age-group wise distribution is statistically significant.

UPHC wise positivity was compared for the first 10 UPHCs with highest positivity (Table-2). The comparison of zone wise positivity with cases (Table-3) shows that the zone most affected by covid19 i.e. Central zone with maximum initial cases had the highest positivity of 28.54%. Thus, the positive antibody status correlates with the documented evidence of high case-load particularly in the first 2 months of the pandemic in Ahmedabad.

Our study included HCWs from field area as well as Covid/Non-Covid hospitals. With 888 sero-positives out of 6509 HCWs, the seropositivity among HCWs is 13.64%. On the other hand, seropositivity among non-HCWs was 18.71%. Seropositivity among non-HCW is significantly higher as compared to the HCWs.

We also tried to compare positivity with days of our study period, which showed wide variation. To remove the effect of daily variation we tried to study the linear trend (Figure-4). We tried to calculate the weekly seropositivity for the 3-week study duration (Table-1), which shows a seropositivity of 13.83%, 20.20% and 22.33% respectively. Zone wise seropositivity & its comparison with cases per 10000 population (Figure-5) and case density (Figure-6) shows that the positivity correlates with the cases in most zones but is strikingly low in Central zone.

## Discussion:

The present study is one of the biggest studies conducted in the world in terms of sample population ratio to find out sero positivity in general population. This study has 4770 samples per million population in comparison to Spanish study where the sample population ratio was 1302 samples per million population and the US study where the sample population ratio was 255 samples per million population.<sup>13,14</sup> With 17.61% seropositivity our results are consistent with other studies showing that even in the areas highly affected by SARS CoV2 during this pandemic, have shown very low level of seropositivity.<sup>8</sup> Antibodies do take some time to develop after an infection, approximately 1 to 3 weeks, with an average of 2 weeks (14 days).<sup>15,16</sup> So, we can say that the rate of antibody positivity reflects the case scenario about 14 days prior to the study. A national seroprevalence study from India, completed a few days before the present study, documented the national seroprevalence of just 0.73% [95% Confidence Interval (CI) 0.34-1.13]. The vast difference reflected in our study, justify our statement for the situation in Ahmedabad during the initial 3 months of the pandemic.<sup>17</sup> On the other hand, the study by ICMR found 55% seroprevalence in the containment zone.<sup>10</sup>

The difference in the positivity for both the sex group is statistically not significant and this finding is similar to other studies from Spain & USA where the studies have found no significant difference between the genders for covid19 antibodies.<sup>7,8,18</sup> Comparatively less samples with wide variation in positivity at the extremes of age group i.e. 0-9 years and 90-99 years for both the gender groups may be the reason for having a statistically significance difference for the age group wise seropositivity. Majority of the HCWs in the study were from the field area – working at the UPHCs. A statistically significant low sero-positivity among HCW as compared to non-HCW

indicate that they are better protected as compared to general population, particularly when the epidemic has progressed to infect a large number of people from general population.

Our results also show that the UPHCs with high number of cases have higher positivity. However, UPHCs with high number of cases in the early phase of the pandemic have low seropositivity as compared to the UPHCs with higher number of cases during the later part of the pandemic (with more cases in the recent past). In Ahmedabad, the initial cases were more from the central zone followed by south zone. Then, gradually due to extensive containment measures the cases from Central zone reduced and cases started appearing more from the East and North zones. It can be seen that the highest seropositivity was found in the individuals from East Zone and North Zone. This is on a slightly higher side than that from the UPHCs of the Central Zone, which had the overall highest number of cases. Strikingly the worst and first affected UPHCs of the Central zone have lesser positivity. The first 8 UPHCs with the highest positivity are from North Zone & East Zone (Having higher cases in the later part of the pandemic period so far) than that of Central zone (having maximum population density and highest cases in the very first phase of pandemic period). This might be pointing towards the fact that the immunity developed after a successful recovery from Covid19 infection may not be lasting enough. Although the same is also reported by other studies, we need more studies and longer follow up to cross verify this aspect and to bring out the scientific fact related with the post-covid immunity.<sup>19-21</sup>

Day to day variation in positivity may be dependent on the proportion of different population groups (e.g. cases, contacts, super spreaders, symptomatic, asymptomatic, HCWs etc.) covered on that day. However, the trend suggest that the positivity slowly increases with time. The same is

the conclusion from the week-wise positivity calculated for the 3 weeks study duration. Both of these findings show that the positivity increase with time & thus correlates with increasing number of cumulative cases.

When we compare cases per million population with sero positivity (Table-3, Figure-5) it was found that most affected central zone with highest cases (5743 per million population) also had highest seropositivity of 28.54%. However, the areas of recent infection, like North Zone and East zone, have higher sero positivity (27.41% & 23.44% respectively) despite having lower cases (2649 & 1777 cases per million population respectively) than the South Zone. The comparative low positivity as compared to high case per million population may be largely due to the sampling method followed, which was independent of the cases from that zone. We also tried to compare the case density (cases per square Km) with sero positivity (Table-3, Figure-6). Here also the findings suggest that the first and worst affected central zone with highest case density had highest seropositivity. Areas of recent infection like North Zone and East zone have higher sero positivity of 27.41% and 23.44% respectively (much higher than that of south zone, i.e. 16.81%) despite having case density comparative with that of south zone (44.5 and 34.6 cases per square Km respectively, as against 37.0 cases per square Km in South Zone). Both this comparison indicates the possibility of vanishing positivity with the passing of time. However, we need more in-depth scientific research to find out the reason behind this paradox.

Looking at the positivity rate at the Covid/Non-Covid hospitals, it is observed that the highest positivity (12.84%) is seen at the SVP hospital which is a dedicated Covid hospital right from the start of the corona pandemic in the city. The overall positivity of 12.84% is still lower than that of

the crude positivity of the general population i.e. 17.61%. This may be due to the fact that all the HCWs in a dedicated Covid hospital are very well protected with PPE & other safety measures.

LG hospital is a non-Covid hospital attached with another medical college run by the AMC. The positivity rate at LG is also quite similar to SVP hospital with 12.80% positivity. Although LG hospital is not a dedicated covid hospital, still the positivity rate here is quite similar to that of the covid hospital. Inspite of being a non-Covid hospital, there were multiple occasions in the previous 2 months when large number of HCWs turned out to be positive & many HCWs were isolated/quarantined. A lot of patients from the containment zone reported here for non-Covid complains but were tested & reported positive. The authorities were even forced to close down the hospital once for about 7 days for improving the sanitization measures and improving the implementation of various SOPs. As compared to the above two hospitals, the other non-Covid hospital (SCL Hospital) did not have many Covid cases and the low positivity (3.09%) at this hospital also correlate with the data.

### **Limitation of our study:**

The study followed convenience sampling at the level of UPHC. Some of the collected data (e.g. symptomatology of the enrolled individual) was not completely available for data analysis.

### **Conclusion:**

This study to assess the seropositivity during the Covid19 pandemic from Ahmedabad, India is one of the first few population-based study from India with a large sample size and a very high sample-population ratio. As of June 2020, the level of Covid-19 seropositivity in Ahmedabad city, India is 17.61%. In view of these findings with the absence of an evidence of lifelong immunity after Covid-19 infection, it can be concluded that the population of Ahmedabad is still largely susceptible. As of now, we cannot rely on this level of immunity to protect and the preventive measures need to be strongly relied upon till an effective vaccine is provided to the people at large. There is no gender difference in sero-positivity but the seropositivity is significantly associated with the risk of covid-19 infection in the area. Although the sero-positivity slowly increases with time there are also indications that these IgG may not be long lasting. Further in-depth scientific studies are required to give more insight for the future predictions.

**Author Contributions:**

Dr. Bhavin Solanki (BS) under the guidance of Dr. OM Prakash (OP) planned and carried out the sero-surveillance. Testing of the samples and reporting was managed by Dr. Sanjay Rathod (SR), Dr. Anil Rajput (AR) and Dr. Toral Trivedi (TT) under the leadership of Dr. Mina Kadam (MK). Data analysis was carried out by Dr. Jay Sheth (JS), Dr. Sheetal Vyas (SV), Dr. Aparajita Shukla (ASh), Dr. Bhavin Joshi (BJ), Dr. Vaibhav Ramanuj (VR) and Dr. Anand Solanki (ASo) while the statistical analysis was done by Dr. Hemant Tiwari (HT). Primary manuscript was prepared by Dr. Om Prakash (OP) and Dr. Jay Sheth (JS) and equally contributed by all the other co-authors. All authors contributed to the interpretation of data and approved the final manuscript after critical review.

**Declaration of interests:**

We declare no competing interests.

**Funding:** Health Department of Ahmedabad Municipal Corporation carried out the study as part of Covid19 pandemic response. There is no external funding

**Data sharing:**

Our data are accessible to researchers upon reasonable request for data sharing to the corresponding author.

**Ethical permission:**

We have received permission for the study from the Institutional Review Board (IRB) of the AMC MET Medical College, Ahmedabad.

### **Acknowledgments:**

We are extremely thankful to respected Dr. Rajiv Kumar Gupta, IAS (Additional Chief Secretary, Government of Gujarat) and Mr. Mukesh Kumar, IAS (Municipal Commissioner, Ahmedabad) for their whole hearted support. This study would not have been possible without the financial support from the authorities of Ahmedabad Municipal Corporation. We acknowledge the full support from the field level health care workers (Corona warriors) who put in great efforts to perform their duties as well as sample collection after informed verbal consent particularly in a Covid-19 pandemic situation. All the Zonal Deputy Health Officers, Deputy Health Officer (Epidemic), Assistant Health Officers and Medical officers of the Urban Primary Health Centers extended full support in conducting the sero-surveillance. We are thankful to all the medical and paramedical support staff posted at the laboratories for their contribution in timely testing such large number of samples with accuracy and quality. Finally, we are indebted to all the patients including health care workers whose willingness and support has generated the much-desired data for the study.

Table-1 Analysis of Covid19 sero-survey positivity

	Female			Male			Total			95% CI	P Value
	Results	Positive	% Positivity	Results	Positive	% Positivity	Results	Positive	% Positivity		
Gender	13847	2489	17.98	16044	2774	17.29	29891	5263	17.61	17.18-18.04	P>0.05
Age group											
0-9	39	3	7.69	53	14	26.42	92	17	18.48	11.15-27.93	P < 0.001
10-19	552	92	16.67	687	106	15.43	1239	198	15.98	14.05-18.13	
20-29	4013	684	17.04	3665	518	14.13	7678	1202	15.66	14.86-16.49	
30-39	3424	569	16.62	3910	636	16.27	7334	1205	16.43	15.60-17.30	
40-49	2837	528	18.61	3162	668	21.13	5999	1196	19.94	18.95-20.97	
50-59	1749	356	20.35	2608	499	19.13	4357	855	19.62	18.47-20.83	
60-69	889	189	21.26	1356	222	16.37	2245	411	18.31	16.76-19.96	
70-79	291	60	20.62	505	95	18.81	796	155	19.47	16.87-22.37	
80-89	49	8	16.33	93	14	15.05	142	22	15.49	9.97-22.51	
90-99	4	0	0	5	2	40.00	9	2	22.22	2.81-60.01	
Zone											
CZ	1876	567	30.22	1897	510	26.88	3773	1077	28.54	27.13-30.01	P < 0.001
NZ	2222	579	26.06	2576	736	28.57	4798	1315	27.41	26.16-28.69	
EZ	1630	378	23.19	2039	482	23.64	3669	860	23.44	22.10-24.84	
SZ	1564	278	17.77	1649	262	15.89	3213	540	16.81	15.55-18.14	
SWZ	1145	167	14.59	1838	235	12.79	2983	402	13.48	12.30-14.75	
WZ	3193	351	10.99	3111	310	9.96	6304	661	10.49	09.75-11.27	
NWZ	1359	76	5.59	2303	160	6.95	3662	236	6.44	05.69-07.29	
Hospital											
SVP (Covid)	339	37	10.91	331	49	14.80	670	86	12.84	10.51-15.58	P < 0.001
LG	418	55	13.16	207	25	12.08	625	80	12.80	10.41-15.65	
SCL	101	1	0.99	93	5	5.38	194	6	3.09	01.14-06.61	
Study week											
Week1	6535	942	14.41	6654	882	13.26	13189	1824	13.83	13.25-14.43	P < 0.001
Week2	5840	1208	20.68	7826	1553	19.84	13666	2761	20.20	19.54-20.88	
Week3	1472	339	23.03	1564	339	21.68	3036	678	22.33	20.89-23.85	
Category											
NonHCW	10114	2003	19.80	13268	2372	17.88	23382	4375	18.71	18.22-19.22	P < 0.001
HCW	3733	486	13.02	2776	402	14.48	6509	888	13.64	12.83-14.50	

Table-2 Top ten UPHC with highest seropositivity results

UPHC of AMC	Total	Results	Positive	Positivity
MEGHANINAGAR (NZ)	119	119	52	43.70
GOMTIPUR (EZ)	308	300	118	39.33
RAJPUR (EZ)	383	383	138	36.03
NARODA ROAD (NZ)	170	170	61	35.88
INDIACOLONY (NZ)	806	802	280	34.91
BAPUNAGAR (NZ)	316	316	110	34.81
KUBERNAGAR (NZ)	480	477	166	34.80
SARASPUR-RAKHIAL (NZ)	401	401	127	31.67
JAMALPUR (CZ)	429	428	135	31.54
SHAHPUR (CZ)	394	394	124	31.47

Table-3 Zone wise comparison of seropositivity and Covid19 cases

Zone	Population	Area (Sq Km)	Cases (As on June 15, 2020)	Case Density (Cases /Sq Km)	Cases per 10000 population	Results	positive	Percent positivity
CZ	683089	18.33	3923	214.0	57.43	3773	1077	28.54
SZ	1081996	88.65	3276	37.0	30.28	3213	540	16.81
NZ	1090409	64.92	2889	44.5	26.49	4798	1315	27.41
EZ	1425254	73.2	2532	34.6	17.77	3669	860	23.44
WZ	1302500	65.68	2357	35.9	18.10	6304	661	10.49
SWZ	524970	61.86	871	14.1	16.59	2983	402	13.48
NWZ	807539	91.36	772	8.5	9.56	3662	236	6.44

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5 Figure-2 Age group wise Sero-positivity  
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7 Figure-3 Age group & sex wise Sero-positivity  
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9 Figure-4 Day wise Sero-Positivity  
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11 Figure-5 Comparison of seroprevalence with zone wise cases  
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13 Figure-6 Comparison of Seroprevalence with case density  
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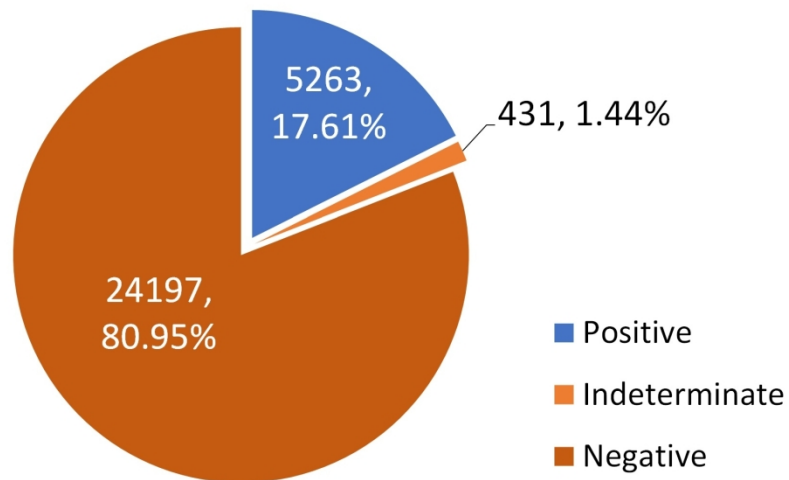
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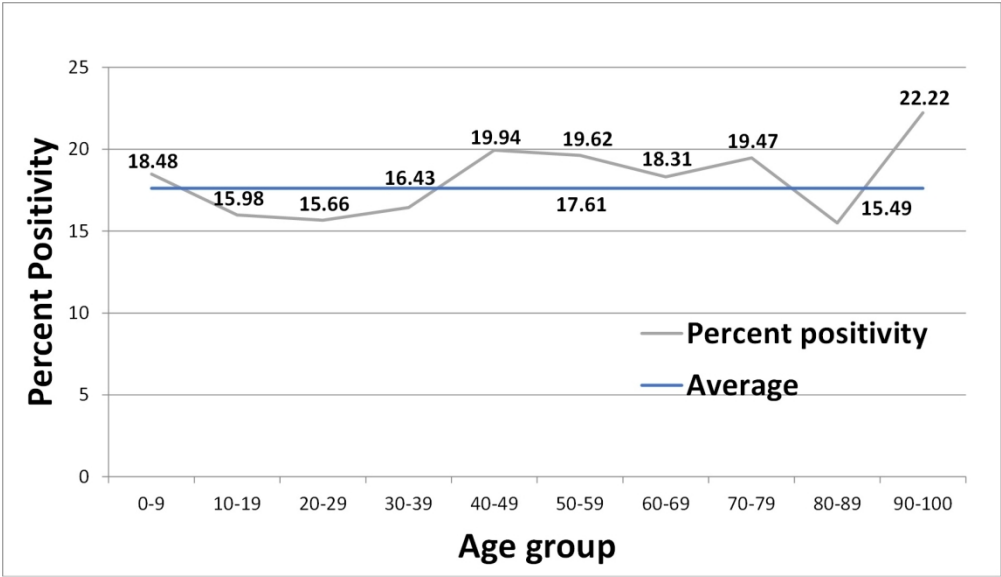
21. Jason Rosado, Stéphane Pelleau, Charlotte Cockram, Sarah Merkling, Narimane Nekkab, et al. Serological signatures of SARS-CoV-2 infection: Implications for antibody-based diagnostics. 2020. {pasteur-02569149v2}

## Result of Sero-surveillance



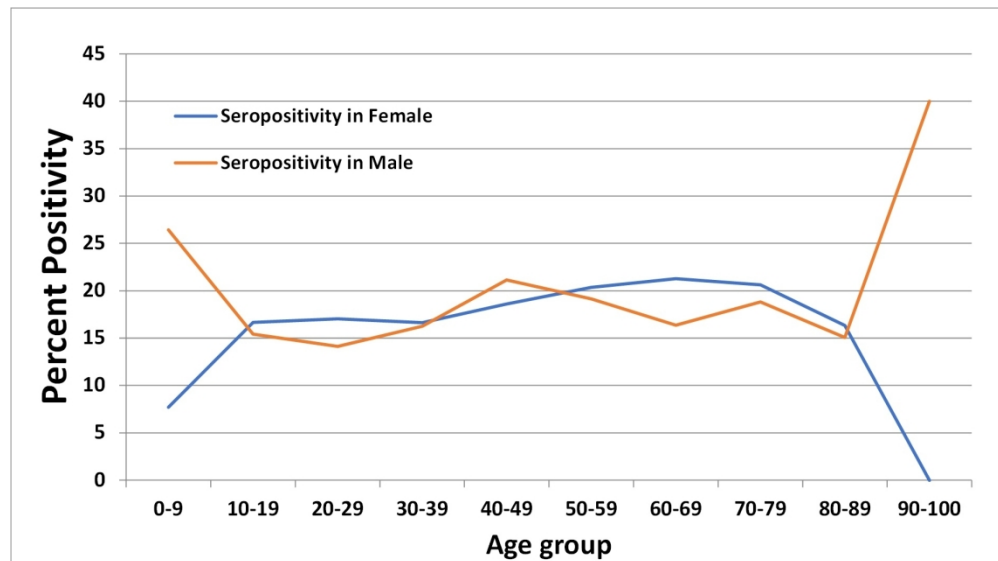
Result of Sero-surveillance

175x109mm (300 x 300 DPI)



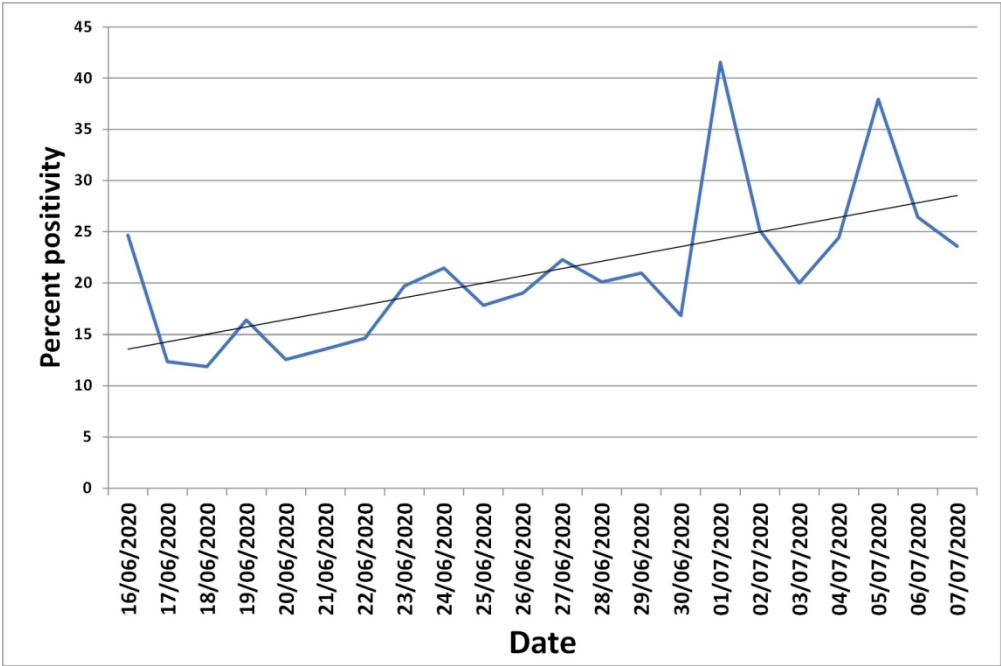
Age group wise Sero-positivity

156x90mm (300 x 300 DPI)



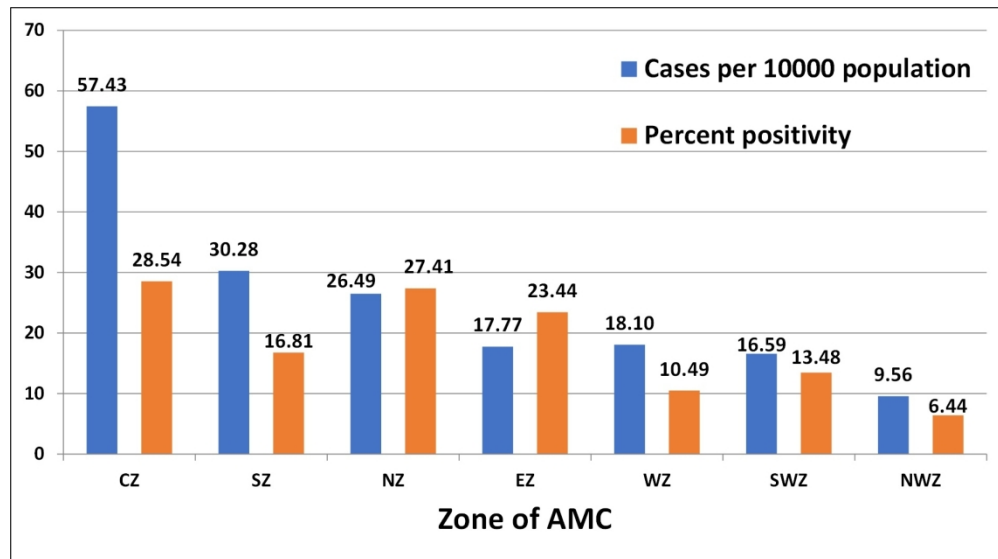
Age group & sex wise Sero-positivity

161x90mm (300 x 300 DPI)



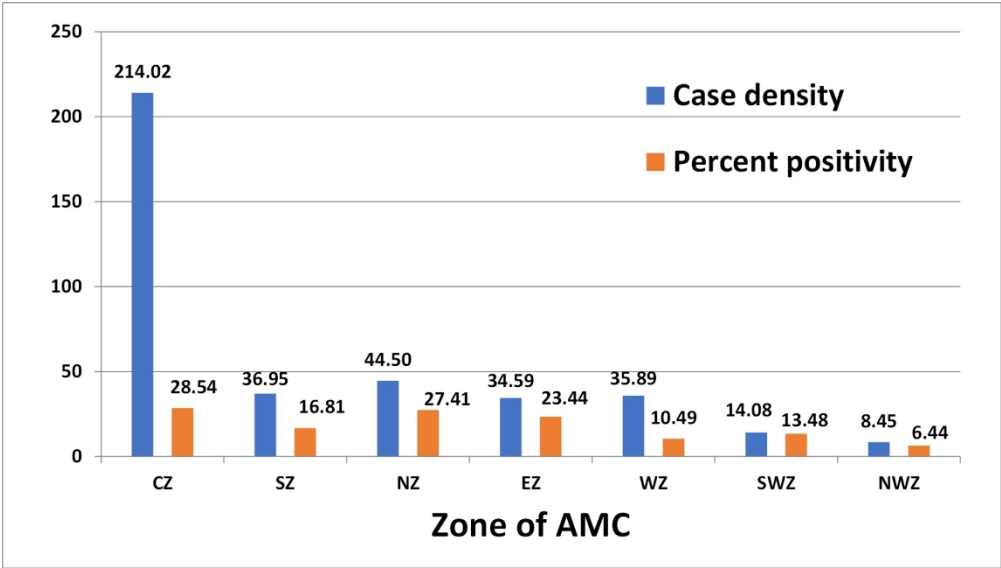
Day wise Sero-Positivity

135x90mm (300 x 300 DPI)



Comparison of seroprevalence with zonewise cases

161x90mm (300 x 300 DPI)



Comparison of Seroprevalence with case density

176x99mm (300 x 300 DPI)

# Reporting checklist for cross sectional study.

Based on the STROBE cross sectional guidelines.

## Instructions to authors

Complete this checklist by entering the page numbers from your manuscript where readers will find each of the items listed below.

Your article may not currently address all the items on the checklist. Please modify your text to include the missing information. If you are certain that an item does not apply, please write "n/a" and provide a short explanation.

Upload your completed checklist as an extra file when you submit to a journal.

In your methods section, say that you used the STROBE cross sectional reporting guidelines, and cite them as:

von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. The Strengthening of Reporting of Observational Studies in Epidemiology (STROBE) Statement: guidelines for reporting observational studies.

			Page
Reporting Item			Number
<b>Title and abstract</b>			
Title	<a href="#">#1a</a>	Indicate the study's design with a commonly used term in the title or the abstract	1

1	Abstract	<a href="#">#1b</a>	Provide in the abstract an informative and balanced summary	3
2			of what was done and what was found	
3				
4				
5				
6	<b>Introduction</b>			
7				
8				
9				
10	Background /	<a href="#">#2</a>	Explain the scientific background and rationale for the	5
11	rationale		investigation being reported	
12				
13				
14				
15	Objectives	<a href="#">#3</a>	State specific objectives, including any prespecified	6
16			hypotheses	
17				
18				
19				
20	<b>Methods</b>			
21				
22				
23	Study design	<a href="#">#4</a>	Present key elements of study design early in the paper	7
24				
25				
26	Setting	<a href="#">#5</a>	Describe the setting, locations, and relevant dates, including	7-9
27			periods of recruitment, exposure, follow-up, and data	
28			collection	
29				
30				
31				
32				
33				
34	Eligibility criteria	<a href="#">#6a</a>	Give the eligibility criteria, and the sources and methods of	7
35			selection of participants.	
36				
37				
38				
39		<a href="#">#7</a>	Clearly define all outcomes, exposures, predictors, potential	8-9
40			confounders, and effect modifiers. Give diagnostic criteria, if	
41			applicable	
42				
43				
44				
45				
46				
47	Data sources /	<a href="#">#8</a>	For each variable of interest give sources of data and details	8-9
48	measurement		of methods of assessment (measurement). Describe	
49			comparability of assessment methods if there is more than	
50			one group. Give information separately for for exposed and	
51			unexposed groups if applicable.	
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Bias	<a href="#">#9</a>	Describe any efforts to address potential sources of bias	8
Study size	<a href="#">#10</a>	Explain how the study size was arrived at	n/a
Quantitative variables	<a href="#">#11</a>	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen, and why	n/a
Statistical methods	<a href="#">#12a</a>	Describe all statistical methods, including those used to control for confounding	20
Statistical methods	<a href="#">#12b</a>	Describe any methods used to examine subgroups and interactions	n/a
Statistical methods	<a href="#">#12c</a>	Explain how missing data were addressed	n/a
Statistical methods	<a href="#">#12d</a>	If applicable, describe analytical methods taking account of sampling strategy	n/a
Statistical methods	<a href="#">#12e</a>	Describe any sensitivity analyses	n/a
<b>Results</b>			
Participants	<a href="#">#13a</a>	Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed. Give information separately for exposed and unexposed groups if applicable.	11
Participants	<a href="#">#13b</a>	Give reasons for non-participation at each stage	n/a

Participants	<a href="#">#13c</a>	Consider use of a flow diagram	n/a
Descriptive data	<a href="#">#14a</a>	Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders. Give information separately for exposed and unexposed groups if applicable.	11
Descriptive data	<a href="#">#14b</a>	Indicate number of participants with missing data for each variable of interest	11
Outcome data	<a href="#">#15</a>	Report numbers of outcome events or summary measures. Give information separately for exposed and unexposed groups if applicable.	11-12
Main results	<a href="#">#16a</a>	Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	n/a
Main results	<a href="#">#16b</a>	Report category boundaries when continuous variables were categorized	20
Main results	<a href="#">#16c</a>	If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	<a href="#">#17</a>	Report other analyses done—e.g., analyses of subgroups and interactions, and sensitivity analyses	n/a
<b>Discussion</b>			
Key results	<a href="#">#18</a>	Summarise key results with reference to study objectives	17

Limitations	<a href="#">#19</a>	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias.	17
Interpretation	<a href="#">#20</a>	Give a cautious overall interpretation considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence.	13-16
Generalisability	<a href="#">#21</a>	Discuss the generalisability (external validity) of the study results	13-16
<b>Other Information</b>			
Funding	<a href="#">#22</a>	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	18

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